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Grape and Wine Research and Development Corporation

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THE THREE R'S - ROOTSTOCK, RESISTANCE AND RESILIENCE TO GRAPE PHYLLOXERA



FINAL REPORT to
GRAPE AND WINE RESEARCH and DEVELOPMENT CORPORATION

Project Number: DPI 08/01

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Research Organisation: **DPI-Rutherglen**

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The Three R's - Rootstock, Resistance and Resilience to Grape Phylloxera

A final report on project DPI 08/01

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ABSTRACT

The overall aim of this research project was to focus on four phylloxera management strategies: detection, quarantine, rootstock selection and awareness, in order to minimise the risk of phylloxera spreading within and between quarantine regions in Australia. During the project period up to six selected phylloxera genotypes were screened against selected grapevine rootstocks under laboratory, glasshouse and field conditions. One new phylloxera detection was reported during the research period and this site was monitored to add value to the research outputs. The influence of various physical and chemical factors, on phylloxera survival was determined with a view to developing a modified disinfestation protocol to reduce the risk of phylloxera transfer in white juice. A comparative analysis of phylloxera early detection techniques was conducted which following further evaluation, may lead to an improved targeted surveillance method for phylloxera. Awareness of phylloxera-related issues were enhanced both nationally and internationally, through (i) participation in the Fifth International Grapevine Phylloxera Symposium and other national and international conferences, (ii) facilitation and participation in National Phylloxera Management Workshops, (iii) the project leaders role as Technical Adviser to the National Phylloxera Technical Reference Group and (iv) publication of results in industry and peer reviewed scientific journals and book chapters. In 2010 the project leader was invited by researchers at INRA Bordeaux and the University of Vienna to be the Australian representative on a proposed new international initiative to sequence the genome of Grape Phylloxera (*Daktulosphaira vitifoliae* Fitch). This could ultimately lead to major advancements in future management of grape phylloxera both nationally and internationally. Recommendations on research priority areas for addressing knowledge gaps in phylloxera management are provided.

EXECUTIVE SUMMARY

This report describes the research and extension activities of a GWRDC-funded project (co-funded by DPI-Victoria and PGIBSA) on phylloxera research and management conducted during the period December 2008-December 2011. Increased scientific knowledge of grapevine phylloxera, obtained during the project period was passed on to stakeholders at all levels, both nationally and internationally. The research activities conducted allowed the development of improved management practices particularly through an in-depth understanding of the interactions between phylloxera clonal lineages and rootstocks, leading to improved rootstock recommendations for Australian conditions. Other research activities undertaken included monitoring at a new phylloxera detection site, which improved our understanding of the population dynamics and relative risk of dispersal of a highly virulent phylloxera genetic lineage. A targeted early phylloxera detection system was evaluated in the Yarra Valley region at three field sites. Further scientific validation of this technology over a range of grape-growing regions may ultimately lead to an enhanced surveillance program for phylloxera. An improved awareness of the impact of pH, sugar concentration, cold temperature, sulphur dioxide and white juice on grape phylloxera survival may lead to improved quarantine protocols for white juice disinfestations.

Phylloxera awareness activities were significantly enhanced during the course of the project through a range of extension activities both nationally and internationally by:

- conducting a variety of extension activities including workshops, field days, seminars, industry and media articles, peer-reviewed scientific papers and presentations at international conferences (e.g. Fifth International Grapevine Phylloxera Symposium, Austria) and national conferences (e.g. Australian Wine Industry Technical Conference

(AWITC, Adelaide) and the Australian Society of Viticulture and Oenology symposium (ASVO, Mildura)

- providing training for growers and phylloxera survey teams in the early detection and identification of field symptoms of phylloxera infestations and pre- and post-incursion options management through Annual Phylloxera Identification and Management Workshops.

Recommendations on research priority areas for addressing significant knowledge gaps in phylloxera management, detection and awareness are provided at the end of each individual chapter.

INTRODUCTION

Project Time-frame

This is a report on phylloxera research, development and extension activities carried out from December 2008 to December 2011. The work was funded by the Grape and Wine Research and Development Corporation, the Phylloxera and Grape Industry Board of South Australia and the Department of Primary Industries, Victoria (DPI).

Project Objectives

The key project objectives were to:

1. Ensure rootstocks being used in the Australian viticulture industry have adequate resistance to a broad range of phylloxera clones under field conditions and will remain a sustainable long-term management strategy for phylloxera under Australian conditions.

2. Conduct a comparative analysis of targeted early detection systems under field conditions.

3. Acquire knowledge which would contribute to the development of a revised quarantine protocol for movement of white juice between designated phylloxera quarantine zones.

4. Transfer knowledge of research and development through appropriate industry channels (e.g. NVHSC, GWRDC, PGIBSA, National Phylloxera Identification and Management Workshops, industry and peer-reviewed scientific journals).

Relationship to National Phylloxera Technical Reference Group R and D Priorities (2009-14)

The National Phylloxera Technical Reference Group (NPTRG), following a meeting in May 2008 (Attwood, Victoria), developed a 5-Year Phylloxera Research Plan (2009-2014). The project objectives align with five of the six identified high priority areas as follows:

1. Evaluation of phylloxera genotypes: rootstock relationships – including emerging cool climate rootstocks
2. Phylloxera survival and disinfestation protocols
3. Evaluation and comparison of early detection methods for phylloxera
4. Investigation into factors affecting phylloxera establishment
5. Ongoing advice regarding policy and protocol development and phylloxera management strategies

The project had five distinct R and D components:

Component 1: Monitoring of New Phylloxera Infestations

- Monitoring and risk assessment of specific phylloxera clonal lineages on newly infested commercially ungrafted *Vitis vinifera* vineyards

Component 2: Phylloxera-Rootstock Recommendations

- Monitoring of specific phylloxera clonal lineages on commercially available rootstocks in climatically different viticultural regions to determine (i) risk of transfer from rootstocks (ii) risk of rootstock breakdown and (iii) quality and yield characteristics of selected grafted rootstocks.

- Rootstock screening of cool-climate rootstock hybrids, against six selected phylloxera clonal lineages under laboratory and glasshouse conditions.

Component 3: Quarantine Protocol Validation

In consultation with the National Phylloxera Technical Reference Group and commercial wineries conduct an *'Assessment of phylloxera survival in white juice under simulated 'commercial' winery conditions'*.

The triphasic experiment would determine:

1. Impact of settling duration and cold temperature on survival of phylloxera in white juice.
2. Impact of pH and Baumé on survival of phylloxera in white juice.
3. Impact of free sulphur levels on survival of phylloxera in white juice.

Component 4: Early Detection Comparative Analysis

Over a three-year period the early detection component of the project was to focus on a comparison of 'early' detection techniques at selected 'known' phylloxera-infested field sites and a 'high' risk field site. At each site comparison of the detection technique was to be made including:

1. *Conventional Ground Surveys* (as this technique is currently the industry standard) - conducted in collaboration with DPI Plant Standards ground survey teams.
2. *Targeted Emergence Trapping* - conducted by project staff with extensive experience in phylloxera field monitoring.
3. *Spectral and Electromagnetic Surveying* - conducted in collaboration with University of New England and Wine Network.

4. *Phylloxera-specific DNA soil probe* - conducted in collaboration with CESAR and SARDI.

Component 5: Dissemination of Research Outputs to Industry

Oral and written presentations on all research outputs made through the National Phylloxera Technical Reference Group, National Phylloxera Workshops, industry and scientific journals, and national and international conferences.

Project Aims and Performance Targets

The project objectives and performance targets, as set out in the original proposal, are summarised as follows:

Objectives	**Performance Target**	
Information on new phylloxera infestations	*Study completed at one newly infested field site*	*1*
Information on phylloxera clones on selected cool climate rootstocks under laboratory conditions	*Completion of two laboratory bioassays:* • 2 rootstocks x 6 phylloxera genotypes	*2*
Information on phylloxera clones on selected rootstocks under glasshouse conditions	*Completion of two glasshouse bioassays:* • 3 rootstocks x 6 genotypes	*3*
Information on phylloxera clones on selected rootstocks under field conditions	*Establishment and completion of three seasons of field data collection on phylloxera abundance and rootstock (7 rootstocks) interactions and vine performance at 3 field sites*	*4*
Scientific validation of National Phylloxera Management Protocols	*Completion of phylloxera survival trials:* • Impact of juice temperature and settling duration on phylloxera survival (Trial 1) • Impact of juice pH and Baumé on phylloxera survival (Trial 2) • Impact of juice free sulphur dioxide level on phylloxera survival (Trial 3)	*5*
Comparative analysis of early detection systems	*Data comparison at early detection field trial sites:* • Four detection systems compared	*6*
Knowledge transfer via National Phylloxera Workshops	*Six phylloxera workshops - conducted over three years*	*7*
Published articles on rootstock performance, quarantine and early detection	*3 published scientific articles and 3 industry articles*	*Appendix 1*
Recommendations to industry on rootstocks and quarantine	*Final Report submitted and data provided to National Phylloxera Technical Reference Group (NPTRG)**	

*note the NPTRG was disbanded during the project period

CHAPTER 1

MONITORING OF NEW PHYLLOXERA INFESTATIONS

Output: *Characterisation of the genetics, seasonal abundance and spatial distribution of grapevine phylloxera in a newly detected phylloxera-infested vineyard*

1. Summary

Phylloxera was first detected in mid-January 2010, in a single block of ungrafted *Vitis vinifera* L. in a commercial vineyard near Mansfield, North East Victoria. The initial detection was made by a routine ground survey conducted by a DPI Victoria-Plant Standards led phylloxera surveillance team.

At the infested vineyard a single phylloxera strain was identified and confirmed by DNA typing as being G4. Following discussions between the affected grower and research staff there then followed a single season field-based research study. In this study four blocks of ungrafted *V. vinifera* were designated to monitor the population dynamics, spatial distribution, relative dispersal risk and impact of a single highly virulent phylloxera genotype. An additional four blocks were monitored by vineyard staff using emergence traps.

Phylloxera abundance was assessed by the use of emergence traps at the soil surface and was deemed relatively high compared with previous studies (Powell *et al*., 2003), thereby representing a high risk of transfer. During the monitoring period, late February until late April 2010, phylloxera was detected in three blocks of ungrafted vines. Unfortunately, due to relatively late detection and also resource limitations, monitoring of the infestation with emergence traps started late in the season and therefore peak population abundance (which in the neighbouring King Valley region is between December and January) could not be

determined. However, although peak abundance could not be determined emergence traps did provide evidence of extended spatial distribution of phylloxera within the infested vineyard.

2. Methods

Site details

The study site was located in a commercial vineyard near Mansfield, outside the North East Victoria phylloxera-infested zone (PIZ). Phylloxera was first detected in the vineyard on 19th January 2010. The phylloxera genotype within the study block was characterised, using microsatellite markers (Umina *et al*., 2007), as G4 which is the predominant genotype also found in the neighbouring King Valley region.

A visual canopy assessment was then made in a number of blocks to determine the potential spread of phylloxera within neighbouring blocks and to identify regions for population monitoring using emergence traps (Powell *et al*., 2009).

Soil type

Soil across the vineyard was classified as a darkish brownish grey colour with a sandy clay loam to a clay loam. Soil analysis of the vineyard had been previously conducted by a commercial provider in 2005 (Table 1).

Table 1 Soil analysis of vineyard soil from Mansfield study site

(Source: Elders, 2005)

Analysis	Value	Low	< Opt/ Norm or Mod	Generally Satisfactory	> Opt/ Norm
pH (1:5 Water)	5.20				
pH (1:5 $CaCl_2$)	4.30				
Nitrate nitrogen mg/kg	2.00				
Sulphate sulphur (MCP) mg/kg	15.00				
Phosphorous (Colwell) mg/kg	15.00				
Phosphorous (Oleen) mg/kg	4.98				
Potassium (Amm-acet.) meq/100g	0.47				
Calcium (Amm-acet.) meq/100g	3.20				
Magnesium (Amm-acet.) meq/100g	2.60				
Aluminium (KCl) meq/100g	0.80				
Sodium (Amm-acet.) meq/100g	0.37				
Chloride mg/kg	40.00				
Cation Exch. Cap. Meq/100g	7.44				
Calcium/Magnesium ratio	1.20				
Aluminium saturation %	11.00				
Sodium % of cations (ESP)	5.00				

Experimental design

The trial blocks consisted of ungrafted *V. vinifera,* with a mixture of Chardonnay and Pinot Noir vines. Row spacing was 2.5m and vine spacing was 1.5m. In the monitoring blocks a total of 70 'emergence' traps were utilized. Traps were placed both in the originally detected infested block (designated Block F) and in surrounding blocks where canopy symptoms

indicated the possible presence of phylloxera. Traps were placed randomly throughout two blocks (E and F) of *V. vinifera* cv. Chardonnay and two blocks of *V. vinifera* cv. Pinot Noir (G and H) to ensure that a representative view of the spatial distribution of infestation was obtained (Fig. 1).

In addition vineyard staff also placed and collected emergence traps, as a potential detection technique, in an additional four blocks I, J, M and N, (Fig. 1) where, although phylloxera had not been detected, some potential phylloxera-induced canopy symptoms presented on some vines.

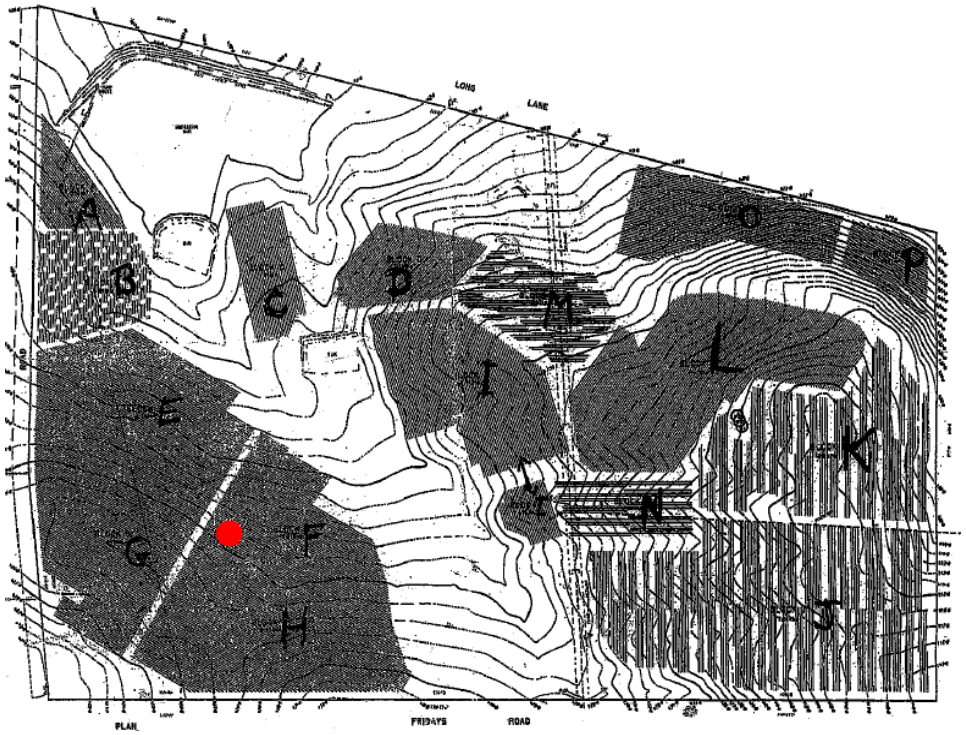


Figure 1. Block layout of phylloxera-infested vineyard, red dot in block F represents the original site of phylloxera detection on 19th January, 2010.

Blocks E-H were monitored fortnightly. Blocks I, J, M and N were monitored monthly. Red dot indicates initial detection point

Trapping and Monitoring

To optimise trap placement a visual assessment of vine vigour was made classifying vines as high or low canopy vigour, or showing signs of stress in the form of foliage yellowing (Fig. 2). To monitor above-ground phylloxera movement, and hence relative risk of transfer,

emergence traps were used. Emergence trapping is a standard protocol developed by DPI-Rutherglen to assess above-ground phylloxera activity, and relative spatial and temporal distribution. The seasonal abundance of phylloxera dispersive stages, first instar 'crawlers' and winged (alate) adults, emerging from below-ground onto the soil surface was measured over the period late-February 2010 to late-April 2010.

Emergence traps, which have been utilized in previous studies (Powell *et al.*, 2000), consisted of translucent plastic containers (5.1L Quadrant (Melbourne, Australia) 22cm in diameter by 17.2cm deep, open at one end and inverted onto the soil surface at a distance of 10cm from the sample vine trunk (Fig. 3). Traps were rinsed with water and fixed flush to the soil surface using metal tent pegs while traps were still moist.

On emergence from the soil, phylloxera dispersive stages were trapped in condensation on the container sides. At either fortnightly or monthly intervals (depending on whether the traps were used for detection or population monitoring) insects were removed by washing the traps with 70% ethanol, thereby killing the phylloxera, and collected in screw-top plastic containers. Traps were then rinsed with tap water and reset. All collected samples were transported under quarantine conditions to the DPI-Rutherglen quarantine laboratory and identified and quantified using a low power binocular microscope. All emergent phylloxera life-stage numbers were recorded.

(a)



(b)



Figure 2. Canopy symptoms, including (a) reduced vigour and (b) foliage yellowing, observed at Mansfield field site were used to determine emergence trap placement on ungrafted *V. vinifera* vines.



Figure 3. Emergence trap placement adjacent to grapevine trunk.

3. Results

Phylloxera emergence and genetic characterisation

Of the emergence traps that were placed in blocks E, F, G and H, phylloxera was detected in all blocks except block E. Phylloxera samples found in blocks F (Table 2), G and H were all confirmed to be the same genotype, G4. From the traps placed in blocks I, J, M and N and collected by the vineyard staff in February and April 2010, no positive detection resulted (Table 3). Seasonal monitoring is presented from block F, where vines were monitored across 32 adjacent rows. Phylloxera abundance was highest on the first collection date in February 2010, with a progressive decline in population size after this time point (Fig. 4; Table 2).

Phylloxera dispersive stages, crawlers and alates, were monitored fortnightly from 26th February 2010 until 27th April 2010 inclusive (Fig. 4). During the season, crawlers were the predominant life-stage detected in trap samples and, although the average number of crawlers per trap was relatively small, the maximum phylloxera number collected for any one sampling period on a single vine was over 400 in late February (Table 2). The peak of phylloxera abundance was most likely (based on previous G4 field trials) to have been in December and January.

Unfortunately monitoring at this site could not commence until February therefore the maximum abundance is most likely to be much higher than reported, particularly as in other nearby infested regions (most notably in the King and Ovens Valleys) where G4 phylloxera has been monitored shortly after detection, abundance has been up to several thousand phylloxera per trap (Powell, 2009).

Table 2 Summary of phylloxera crawler numbers detected in Block F using emergence traps

Researcher-based monitoring conducted by trained staff

	Collection dates (2010)				
Phylloxera no.	**26th February**	**15th March**	**29th March**	**13th April**	**27th April**
Average	16.4	5.7	6.7	8.0	3.1
Maximum	463	121	51	223	66
Minimum	0	0	0	0	0

Table 3 Summary of phylloxera crawler numbers detected in four blocks (I, J, M and N) using emergence traps

Farmer-based monitoring conducted by vineyard staff

	Collection date (2010)/phylloxera number	
Block	**26th February**	**13th April**
I	0	0
J	0	0
M	0	0
N	0	0



Figure 4. Average phylloxera crawlers collected in emergence traps at the base of *Vitis vinifera* cv. Chardonnay, at the Mansfield field site, over the 2010 growing season.

4. Discussion

The phylloxera genetic strain G4 has been monitored previously under field conditions in the King Valley and Ovens Valley on ungrafted *V. vinifera* (Powell *et al*., 2003; Powell, 2009). G4 is notoriously vigorous, compared to most other known phylloxera strains, and therefore represents a high risk of transfer, particularly in cool-climate grape-growing regions where environmental conditions favour its development.

This study again confirms that, at least in the cool climate regions, when G4 phylloxera establishes it causes economic damage, and spreads within the vineyard, relatively quickly when compared to other phylloxera genotypes previously studied. Emergence traps proved effective in detecting the presence of phylloxera in two additional blocks where routine ground-surveying had not detected the pest.

5. Recommendations

1. Because of the very high risk of transfer within the infested vineyard monitoring of, as yet uninfested, grapevine blocks is recommended to enable careful planning of a rootstock replanting program using rootstocks that are highly resistant to the G4 phylloxera genetic strain (Chapters 2-4), the only strain detected in this study site. In addition electromagnetic induction (EM38) soil surveys may potentially provide a useful tool to determine where to place future traps for detection (Chapter 6).
2. It is recommended, for any future new incursions, to commence monitoring within a few days of detection where possible and, if sufficient resources are made available, expand the areas of monitoring beyond those immediately adjacent to the known infested block to determine the broader spatial distribution of phylloxera within the affected vineyard, thereby enhancing rapid incursion management.
3. In addition any new incursion areas would be ideal sites at which to further evaluate targeted phylloxera detection protocols (see Chapter 6).

CHAPTER 2

IN VITRO SCREENING FOR PHYLLOXERA RESISTANCE

Output: *Information on phylloxera clones on selected cool climate rootstocks under laboratory conditions*

Summary

A unique triphasic systematic rootstock screening approach, using selected clonal lineages of phylloxera known to occur in Australia, has been developed by DPI-Victoria so that rootstock recommendations for phylloxera resistance applicable to the Australian viticulture industry can be developed (Powell, 2006). Eighty-three different phylloxera genotypes have been identified to-date in Australia (Umina *et al*., 2007), some of which have different virulence levels on different *Vitis* hybrid species (Powell, 2003). Six clonal lineages have been recommended for rootstock screening purposes (Hoffmann, 2003) and a range of, both commercially available and new, rootstock hybrids have now been screened against selected phylloxera strains (Korosi *et al*., 2007; 2009; 2011a, 2011c).

In this chapter the performance of six selected phylloxera genetic strains on *V. vinifera* and commercially available *Vitis* rootstocks was assessed in a series of 12 excised-root *in vitro* laboratory bioassays. In addition, during the course of the project period, screening of several novel rootstock hybrids for phylloxera resistance was also conducted by the project team (results presented in Clingeleffer and Smith, 2011).

The excised root bioassay technique has been used worldwide routinely for phylloxera screening to compare the level of virulence and development of phylloxera 'populations' (i.e. undetermined genetic background) on *Vitis* rootstocks. Using this technique in this study we compared six selected grape phylloxera genetic strains on up to five rootstock types; 420A (*V.*

berlandieri x V. riparia), 101-14 (*V. riparia x V. rupestris*), 3309C (*V. riparia x V. rupestris*), 1103 Paulsen (*V. berlandieri x V. rupestris*) and *V. vinifera* (susceptible control). Based on these assessments rootstocks were classed as resistant, partially resistant, tolerant or susceptible.

Grape phylloxera strains differed in their ability to survive, develop and produce eggs on different rootstock types. Results presented in this and subsequent chapters (3 and 4) have important implications for future rootstock recommendations for resistance to specific grapevine phylloxera clonal lineages. A summary of rootstock ratings for phylloxera resistance based on *in vitro* screening conducted in this and previous projects (Powell 2003; 2006) are presented.

Methods

Plant and insect material

For all bioassays lignified roots of all rootstocks (101-14 (VIC), 420A, 3309C and 1103 Paulsen) and controls, *V. vinifera* cv. Sultana source material were collected from a reference field collection maintained at CSIRO Merbein located in the Western Phylloxera Exclusion Zone (PEZ) of Victoria. A second source of 101-14 was sourced from Dareton in NSW and was designated 101-14 (NSW).

The six phylloxera genotypes designated, G1, G4, G7, G19, G20 and G30, used in the trials were all collected from commercial vineyards in the Nagambie (G1) and North East (G4, G7, G19, G20 and G30) Phylloxera Infested Zones (PIZ's) respectively. Prior to each experiment, populations of each clonal lineage were maintained separately on excised *V. vinifera* cv. Sultana roots (Kingston *et al*., 2007) in a dark controlled environment growth room (25±2°C) at the DPI-Rutherglen Centre under quarantine conditions.

The phylloxera clonal lineages were genotyped at trial commencement (to ensure single phylloxera strains were utilised) and after cessation of each trial (to ensure no cross-

contamination of strains had arisen during the course of the trial). Rootstocks were also DNA typed.

Excised root bioassay procedure

All twelve bioassays presented here were conducted on excised grapevine root pieces based on a modified method of Kingston *et al*. (2007). Treatments (i.e. rootstocks) and control (i.e. *V. vinifera*) were replicated 7-10 times depending on availability of source root material. For each replicate two excised root pieces from the same rootstock or control were placed in a 140 x 25mm Petri-dish lined with filter paper. Twenty, two-to-five day old eggs of known phylloxera strains from pure stock cultures, of single sourced populations, were placed on each root piece.

Petri-dish edges were sealed with Vitifilm™ to avoid cross contamination of genetic strains and stored in the dark in plastic containers at 23^{o}C ± 2^{o}C. After the phylloxera eggs had been introduced to the root pieces, the rate of phylloxera development was assessed weekly by counting of individual life-stages using a lower power binocular microscope.

For each phylloxera strain, initial infestation and screening occurred on separate consecutive days to prevent cross-contamination. All bioassays carried out during the project period are reported here.

A total of four commercially available rootstocks, 420A, 3309C, 101-14 and 1103 Paulsen were screened, based on recommendations from the National Phylloxera Technical Reference Group. In addition several newly developed hybrid rootstocks supplied by CSIRO Merbein were also screened (as reported in Clingeleffer and Smith, 2011).

Statistical Analysis

For all experiments the total number of each phylloxera life-stage surviving up to eight weeks was used for statistical purposes. Statistical analyses were conducted using GenStat 12 for Windows software (Payne *et al*., 2009). Phylloxera life-stage data were analysed using non-

parametric methods at each week of the trial. A Mann-Whitney U (Wilcoxon rank-sum) paired test was used to compare the results between any two rootstocks and phylloxera strains.

Results

G1 Phylloxera

In bioassay one, conducted over an eight-week period, the performance of the G1 phylloxera strain was compared against four rootstock types: 420A, 101-14 (VIC), 101-14 (NSW) and *V. vinifera* cv. Sultana (Fig. 5).

In bioassay two conducted over an eight-week period, the performance of the G1 phylloxera strain was compared against two rootstocks: 3309C and *V. vinifera* cv. Sultana (Fig. 6). As observed in previous trials the G1 phylloxera strain developed to adulthood and produced eggs on *V. vinifera* after 4 weeks in both trials (Figs. 7 and 8).

On rootstock 420A, G1 phylloxera died within one week and therefore did not develop beyond the crawler stage indicating a highly resistant interaction (Fig. 5). On the rootstock 101-14 (sourced from NSW), G1 phylloxera died within two weeks and therefore did not develop beyond the crawler stage also indicating a highly resistant interaction. In contrast 101-14 (sourced from Victoria) G1 phylloxera survived at least 8 weeks, had higher survival rates than the control, developed into adults and produced eggs after 3 weeks (Fig. 7). In contrast on the rootstock 3309C, although G1 phylloxera survived for up to seven weeks (Fig. 6) it did not produce eggs (Fig. 8).

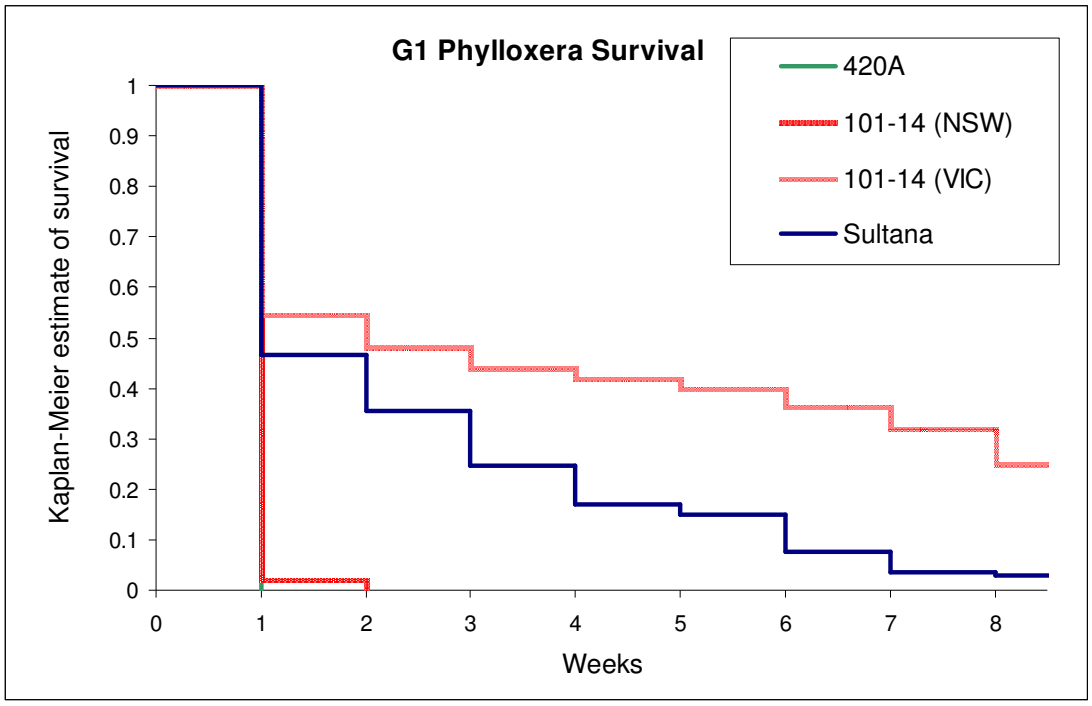


Figure 5. **Excised root bioassay one: Comparison of phylloxera strain G1 survival on rootstocks 420A, 101-14 (VIC), 101-14 (NSW) and *V. vinifera* cv. Sultana.**

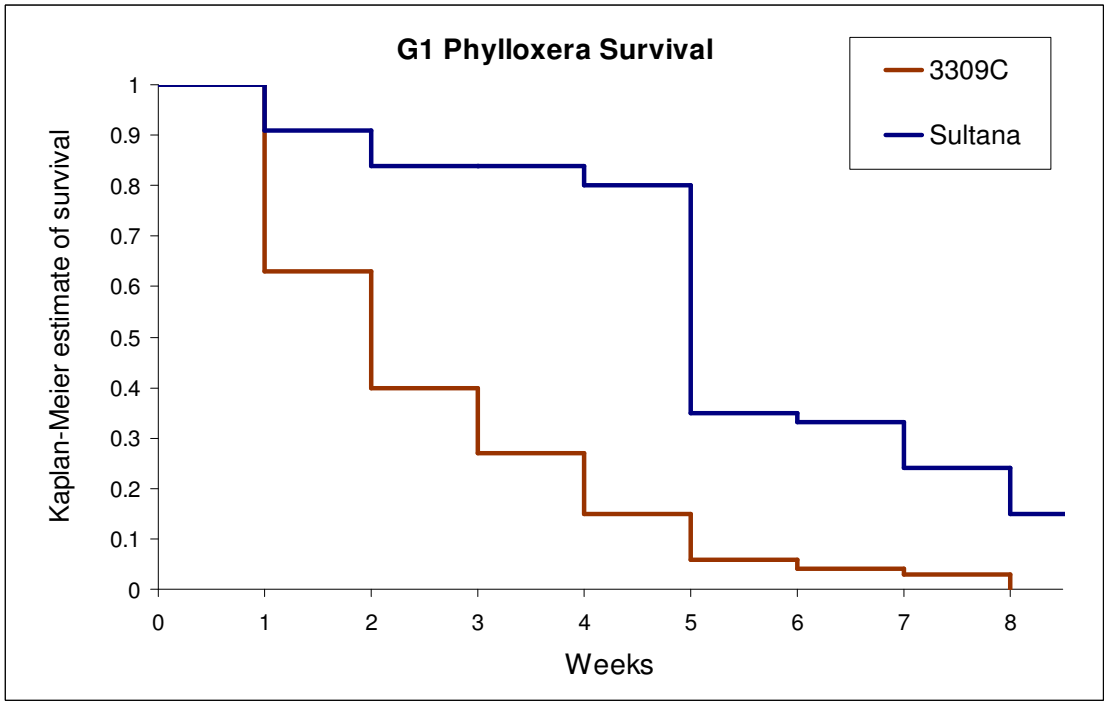


Figure 6. **Excised root bioassay two: Comparison of phylloxera strain G1 survival on rootstocks 3309C and *V. vinifera* cv. Sultana.**

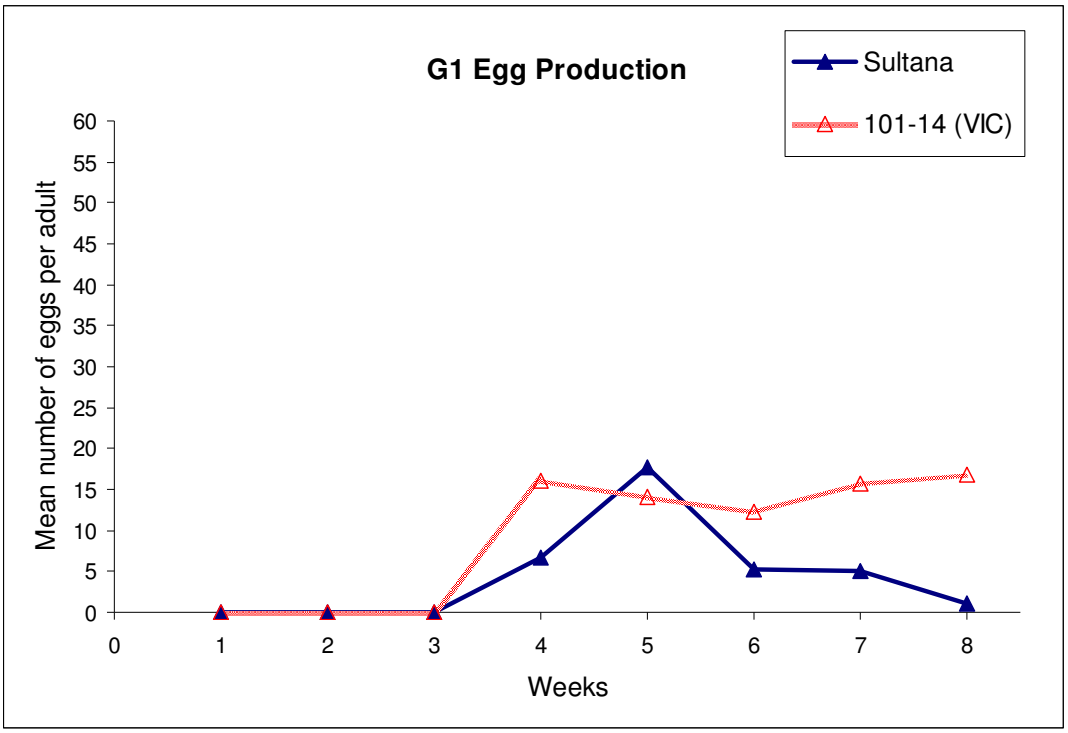


Figure 7. Excised root bioassay one: Egg production of G1 phylloxera strain on *V. vinifera* cv. Sultana. *No egg production was observed on the rootstocks 420A or 101-14 (NSW) during the eight-week trial period.*

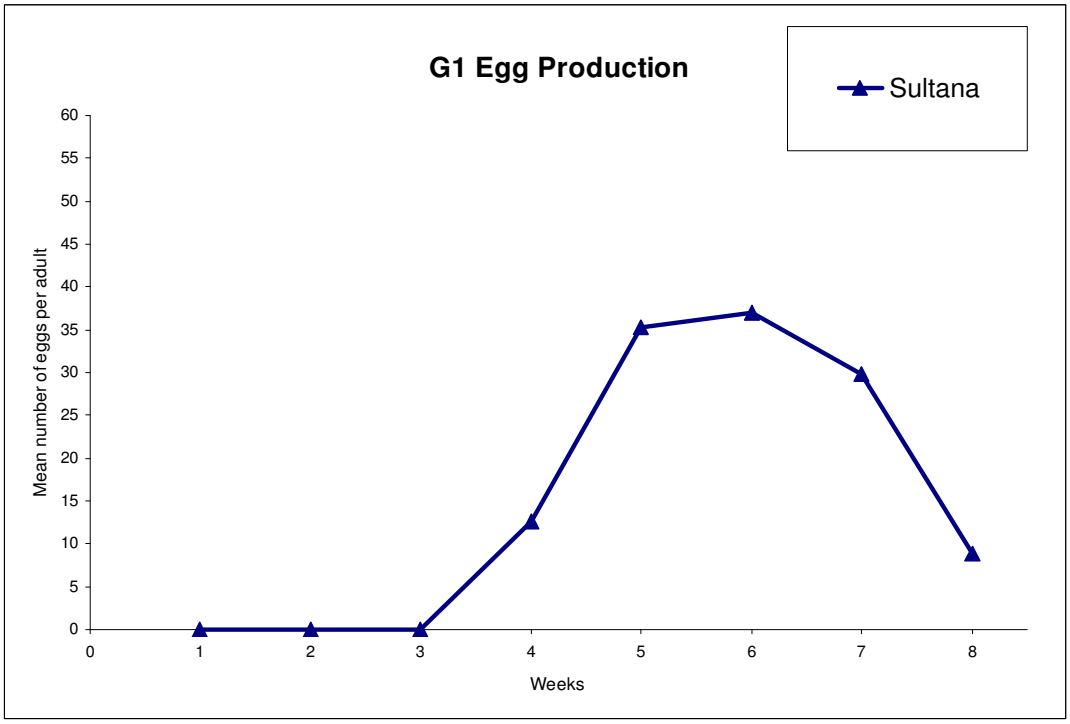


Figure 8. Excised root bioassay two: Egg production of G1 phylloxera strain on *V. vinifera* cv. Sultana. *No egg production was observed on the rootstock 3309C during the eight-week trial period.*

G4 Phylloxera

In bioassay three, conducted over an eight-week period, the performance of the G4 phylloxera strain was compared against five rootstock types: 420A, 101-14 (VIC), 101-14 (NSW), 1103 Paulsen and *V. vinifera* cv. Sultana (Fig. 9).

In bioassay four conducted over an eight-week period, the performance of G4 phylloxera was compared against two rootstock types: 3309C and *V. vinifera* cv. Sultana (Fig. 10).

As observed in previous trials the G4 phylloxera strain developed to adulthood and produced eggs on *V. vinifera* after 4 weeks in both trials (Figs. 11 and 12). Although on 101-14 (sourced from NSW) and 420A G4 phylloxera survived for seven and eight weeks respectively, it did not develop to adulthood or produce eggs. In contrast, 101-14 sourced from Victoria G4 phylloxera survived for at least eight weeks, developed to adulthood and began producing eggs at week 4 (Fig. 11). On the rootstock 1103 Paulsen, G4 phylloxera died within six weeks and again did not develop beyond the crawler stage. On the rootstock 3309C, some G4 phylloxera survived for up to eight weeks yet did not reach the adult stage and hence did not produce eggs.

G7 Phylloxera

In bioassay five, conducted over an eight-week period, the performance of the G7 phylloxera strain was compared against four rootstock types: 420A, 101-14 (NSW), 101-14 (VIC), and *V. vinifera* cv. Sultana (Fig. 13). In bioassay six conducted over an eight-week period, the performance of the phylloxera strain was compared against two rootstock types: 3309C and *V. vinifera* cv. Sultana (Fig. 14).

As observed in previous trials the G7 phylloxera strain developed to adulthood and produced eggs on *V. vinifera* after 4 weeks (Figs. 15 and 16). On the rootstock 420A, G7 phylloxera died within three weeks (Fig. 13), did not develop beyond the crawler stage and consequently no egg production occurred indicating a highly resistant interaction. In contrast on the rootstocks 101-14 (NSW), 101-14 (VIC) and 3309C, G7 phylloxera survived for at least eight weeks (Figs. 13 and 14), reaching the adult stage and although producing less

adults than the control, each surviving adult produced more eggs than the control adults (Figs. 15 and 16). This finding highlighted that both 101-14 and 3309C are tolerant to G7 phylloxera.

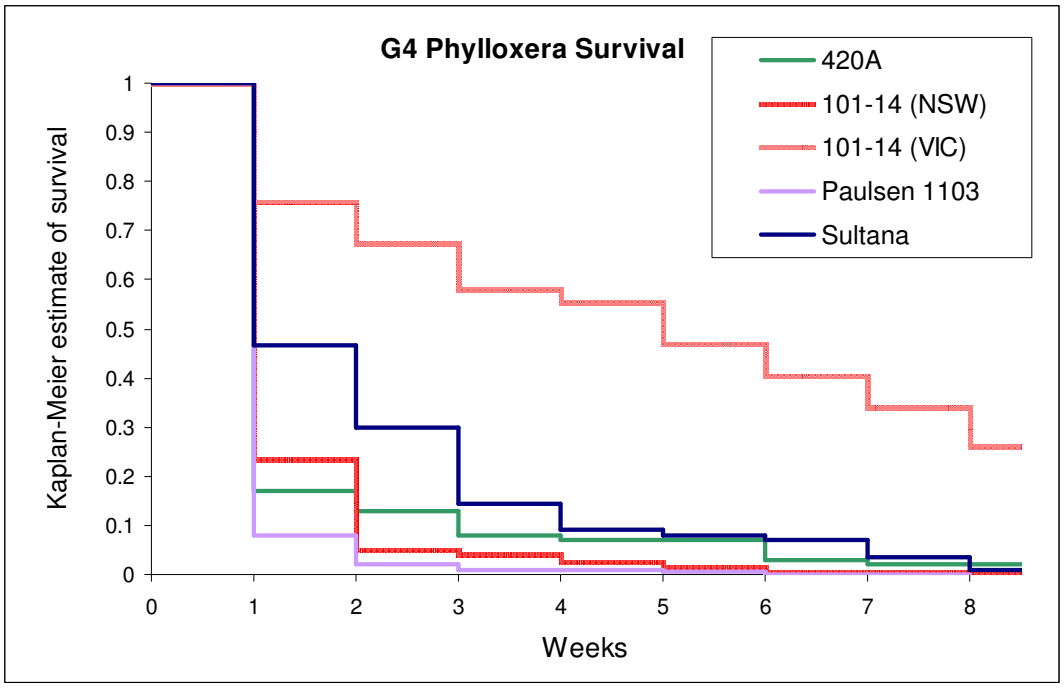


Figure 9. Excised root bioassay three: Comparison of phylloxera strain G4 survival on rootstocks 420A, 101-14, 1103 Paulsen and *V. vinifera* cv. Sultana.

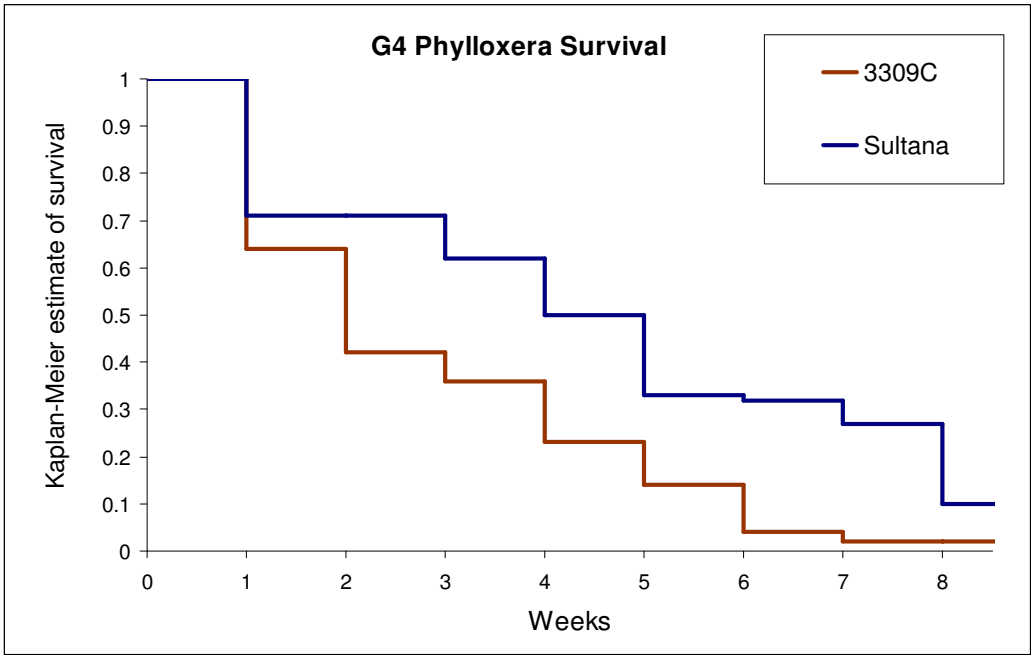


Figure 10. Excised root bioassay four: Comparison of phylloxera strain G4 survival on rootstocks 3309C and *V. vinifera* cv. Sultana.

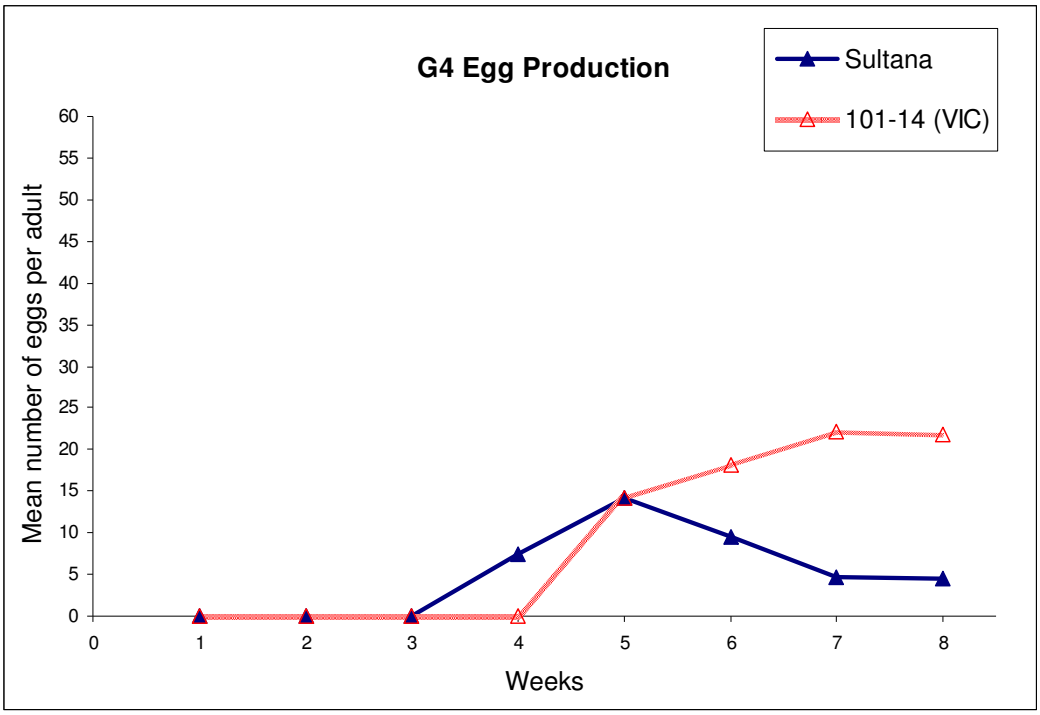


Figure 11. Excised root bioassay three: Egg production of G4 phylloxera strain on *V. vinifera* cv. Sultana.

No egg production was observed on the rootstocks 420A, 101-14 (NSW) or 1103 Paulsen during the eight-week trial period.

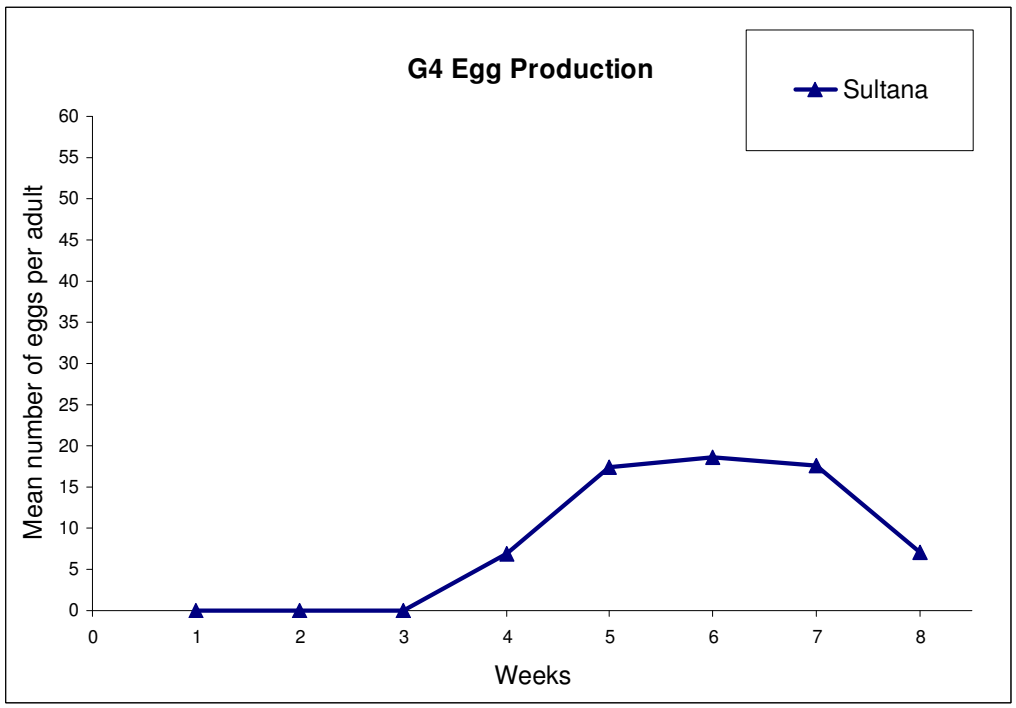


Figure 12. Excised root bioassay four: Egg production of G4 phylloxera strain *V. vinifera* cv. Sultana.

No egg production was observed on the rootstocks 3309C during the eight-week trial period.

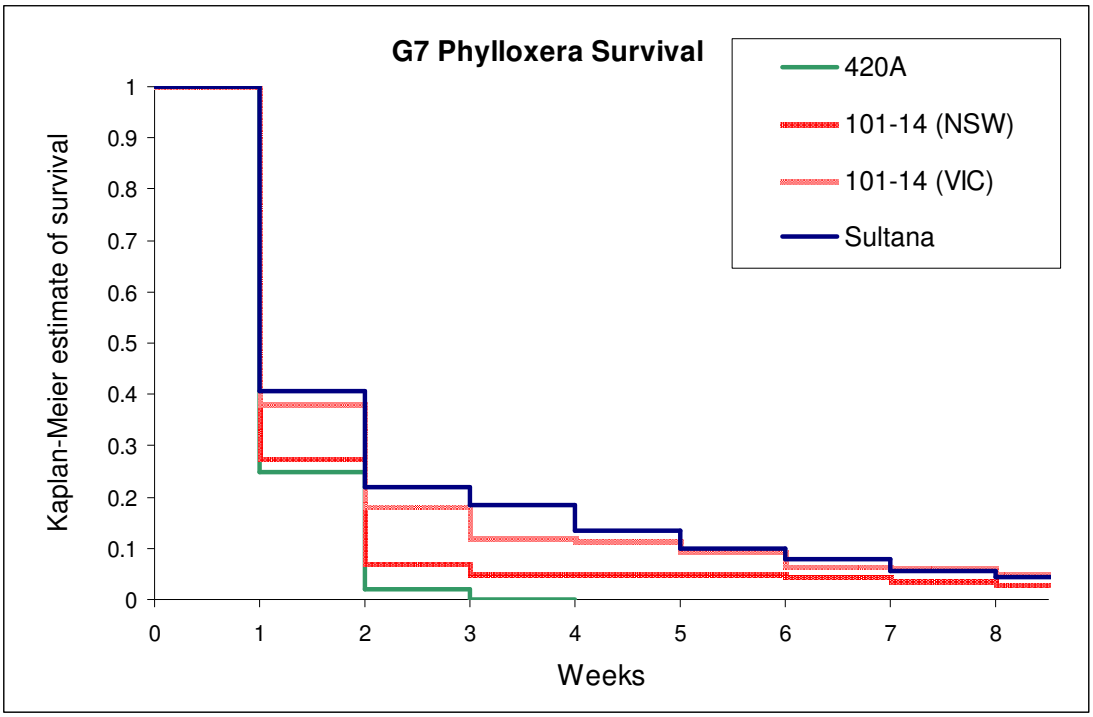


Figure 13. Excised root bioassay five: Comparison of phylloxera strain G7 survival on rootstocks 420A, 101-14 and *V. vinifera* cv. Sultana.

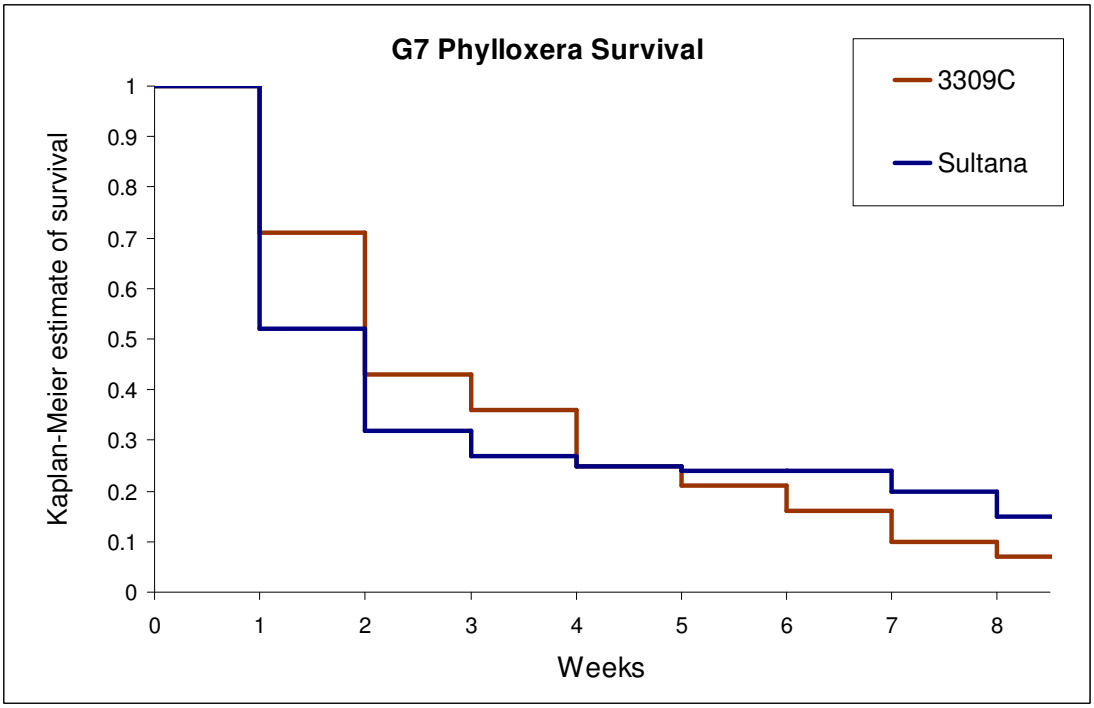


Figure 14. Excised root bioassay six: Comparison of phylloxera strain G7 survival on rootstocks 3309C and *V. vinifera* cv. Sultana.

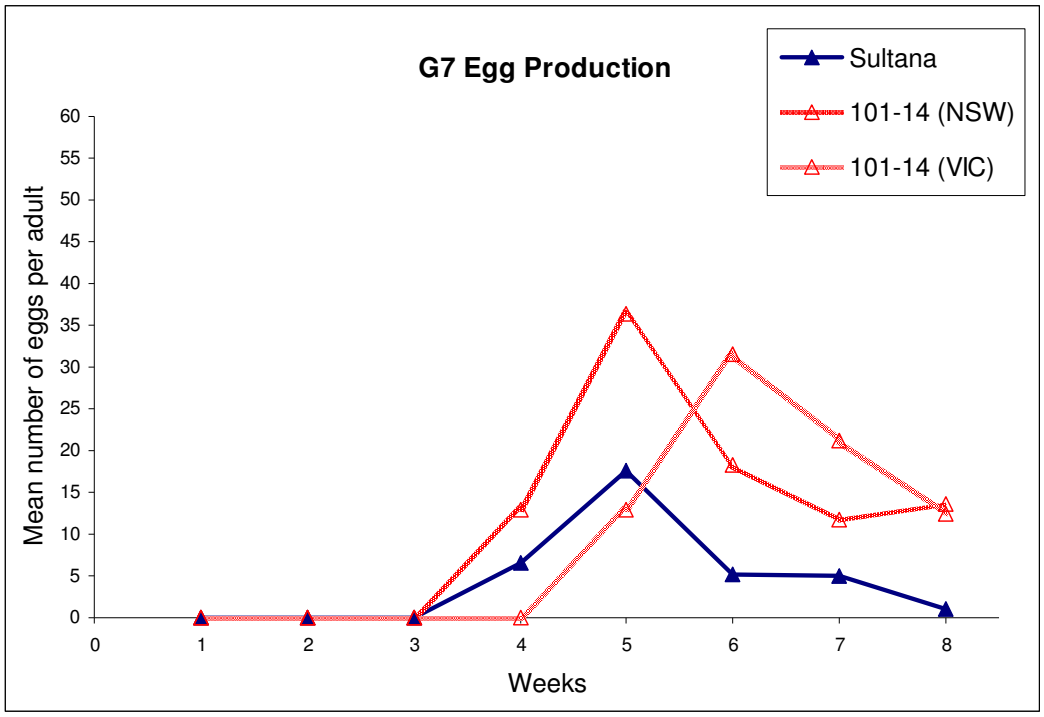


Figure 15. Excised root bioassay five: Egg production of G7 phylloxera strain on 101-14 (NSW), 101-14 (VIC) and *V. vinifera* cv. Sultana.

No egg production was observed on the rootstocks 420A during the eight-week trial period.

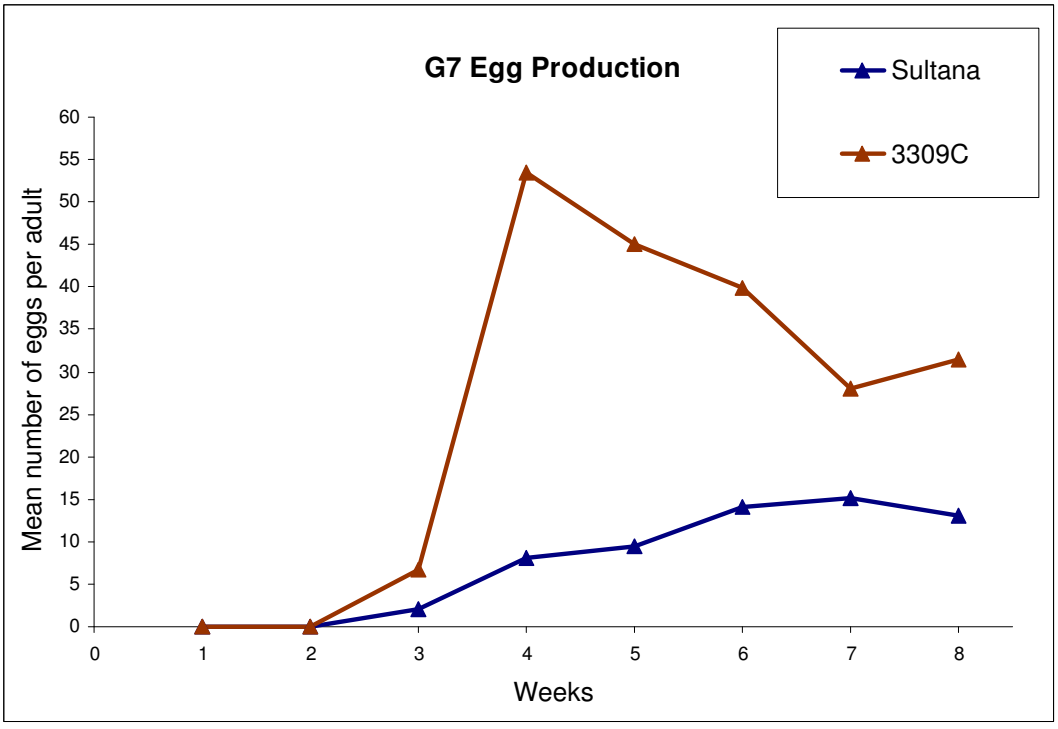


Figure 16. Excised root bioassay six: Egg production of G7 phylloxera strain on 3309C and *V. vinifera* cv. Sultana.

G19 Phylloxera

In bioassay seven, conducted over an eight-week period, the performance of the G19 phylloxera strain was compared against four rootstock types: 420A, 101-14 (NSW), 101-14 (VIC) and *V. vinifera* cv. Sultana (Fig. 17). In bioassay eight conducted over an eight-week period, the performance of the G19 phylloxera strain was compared against two rootstock types, 3309C and *V. vinifera* cv. Sultana (Fig. 18).

As observed in previous trials, G19 phylloxera developed to adulthood and produced eggs on *V. vinifera* after 4 weeks (Fig. 19). On the rootstock 101-14 (NSW), G19 phylloxera died within three weeks, did not develop beyond the crawler stage and consequently no egg production occurred indicating a highly resistant interaction. In contrast on the rootstocks 101-14 (VIC), 420A and 3309C, G19 phylloxera survived for at least eight weeks. G19 phylloxera produced eggs on 1-01-14 (VIC), 3309C and the control. More eggs per adult were produced on 3309C and 101-14 (VIC) compared with the control (Fig. 19), highlighting that both rootstocks are tolerant to G19 phylloxera.

G20 Phylloxera

In bioassay nine, conducted over an eight-week period, the performance of the G20 phylloxera strain was compared against four rootstock types: 420A, 101-14 (VIC), 101-14 (NSW) and *V. vinifera* cv. Sultana (Fig. 20). In bioassay ten conducted over an eight-week period, the performance of the G20 phylloxera strain was compared against two rootstock types, 3309C and *V. vinifera* cv. Sultana (Fig. 21).

As observed in previous trials the G20 phylloxera strain developed to adulthood and produced eggs on *V. vinifera* after 4 weeks (Fig. 22). On the rootstock 101-14 (NSW) and 420A, G20 phylloxera died within three and four weeks respectively weeks and did not develop beyond the crawler stage and consequently no egg production occurred, indicating a highly resistant interaction. In contrast on the rootstocks 3309C and 101-14 (VIC), G20 phylloxera survived for at least eight weeks producing eggs in higher abundance per adult than on the control, thus highlighting that both rootstocks appear tolerant to G20 phylloxera.

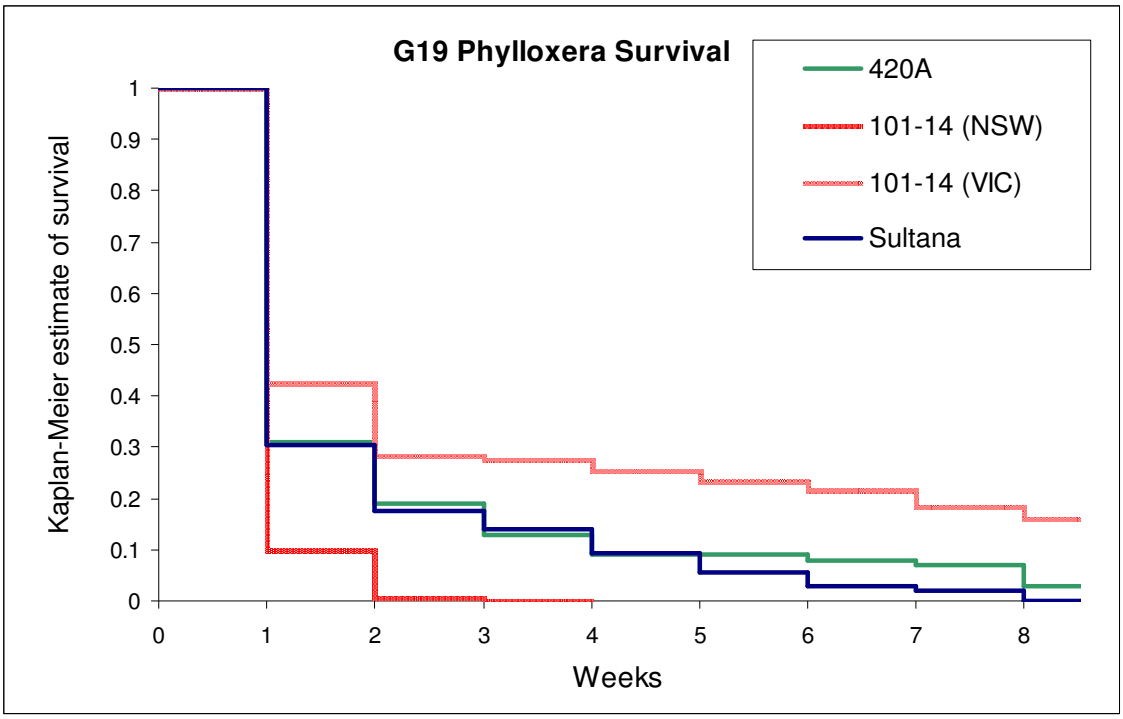


Figure 17. Excised root bioassay seven: Comparison of phylloxera strain G19 survival on rootstocks 420A, 101-14 (NSW), 101-14 (VIC) and *V. vinifera* cv. Sultana.

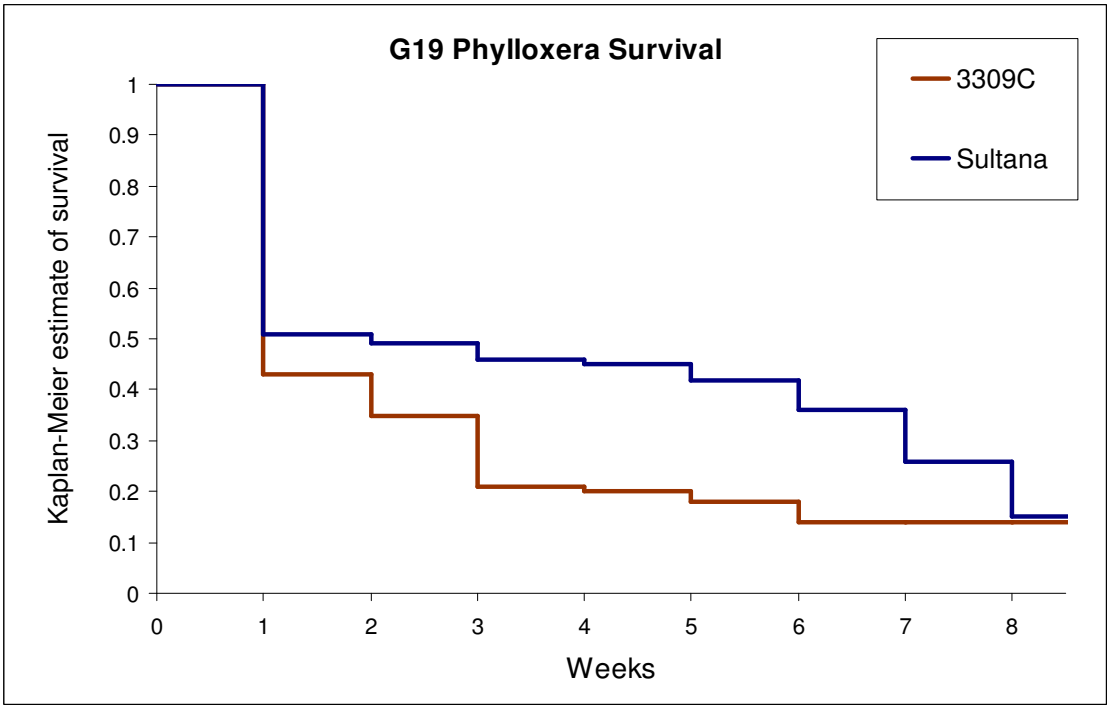


Figure 18. Excised root bioassay eight: Comparison of phylloxera strain G19 survival on rootstocks 3309C and *V. vinifera* cv. Sultana.

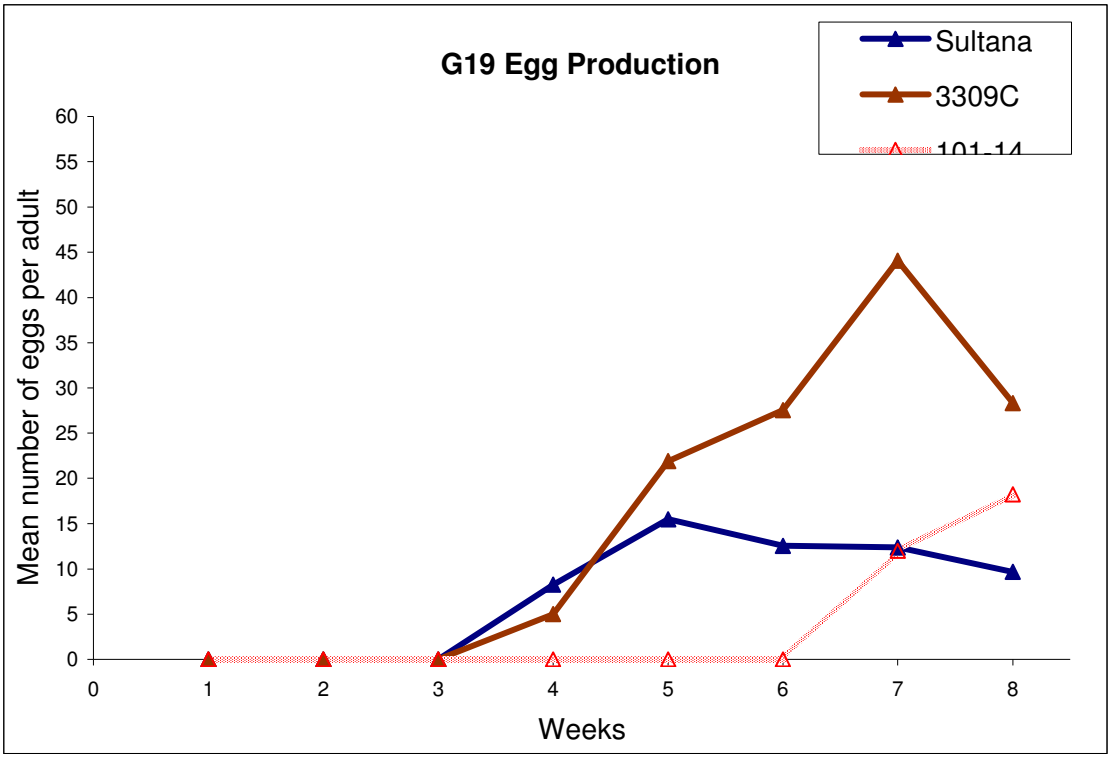


Figure 19. Excised root bioassays seven* and eight: Egg production of G19 phylloxera strain on 3309C, 101-14 (VIC) and *V. vinifera* cv. Sultana.

No egg production was observed on the rootstocks 420A or 101-14 (NSW) during the eight-week trial period.

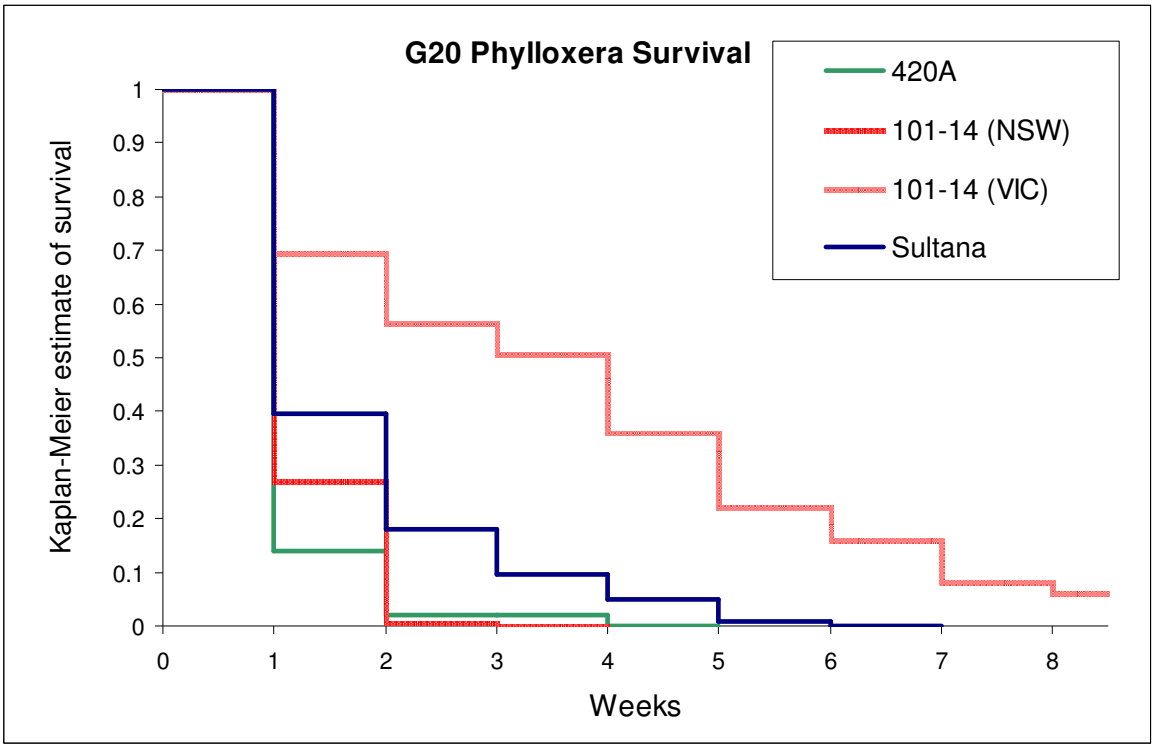


Figure 20. Excised root bioassay nine: Comparison of phylloxera strain G20 survival on rootstocks 420A, 101-14 (NSW), 101-14 (VIC) and *V. vinifera* cv. Sultana.

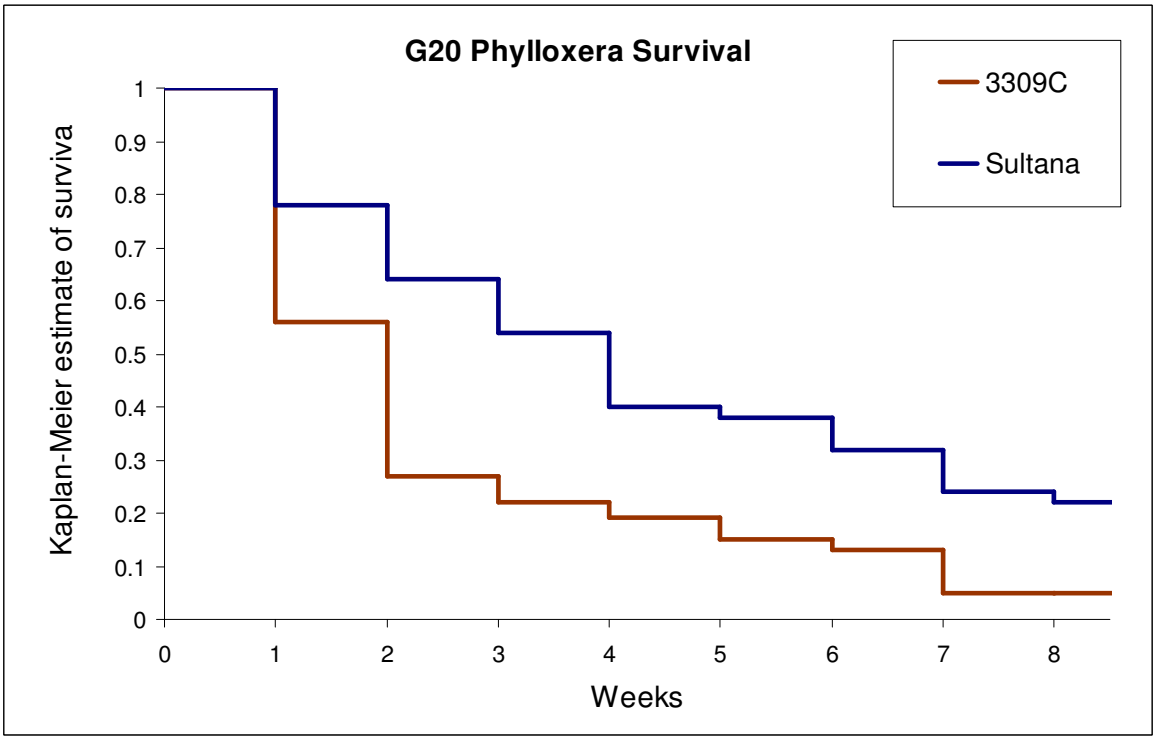


Figure 21. Excised root bioassay ten: Comparison of phylloxera strain G20 survival on rootstocks 3309C and *V. vinifera* cv. Sultana.

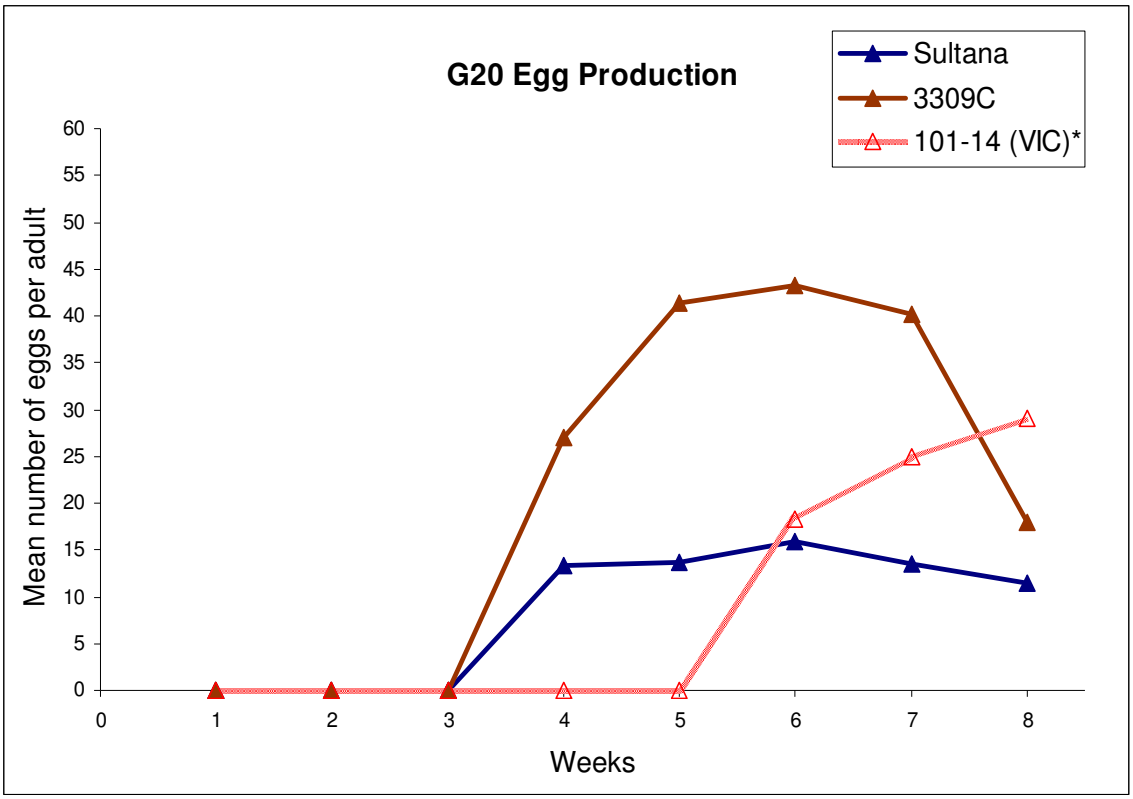


Figure 22. Excised root bioassays nine* and ten: Egg production of G20 phylloxera strain on 3309C, 101-14 (VIC) and *V. vinifera* cv. Sultana.
No egg production was observed on the rootstocks 420A or 101-14 (NSW) during the eight-week trial periods.

G30 Phylloxera

In bioassay eleven, conducted over an eight-week period, the performance of the G30 phylloxera strain was compared against four rootstock types: 420A, 101-14 (VIC), 101 (NSW) and *V. vinifera* cv. Sultana (Fig. 23). In bioassay twelve, conducted over an eight-week period, the performance of the G30 phylloxera strain was compared against two rootstock types: 3309C and *V. vinifera* cv. Sultana (Fig. 24).

As observed in previous trials the G30 phylloxera strain developed to adulthood and produced eggs on *V. vinifera* after 4 weeks (Fig. 25). On the rootstocks 101-14 (VIC) and 420A, G30 phylloxera died within three and four weeks respectively. G30 did not develop beyond the crawler stage and consequently no egg production occurred indicating a highly resistant interaction. In contrast on the rootstock 3309C and 101-14 (NSW), G30 phylloxera survived for at least eight weeks. Eggs were produced in higher abundance per adult on

3309C and 101-14 (VIC) than on the control, highlighting that both 3309C and 101-14 (VIC) are tolerant to G30 phylloxera.

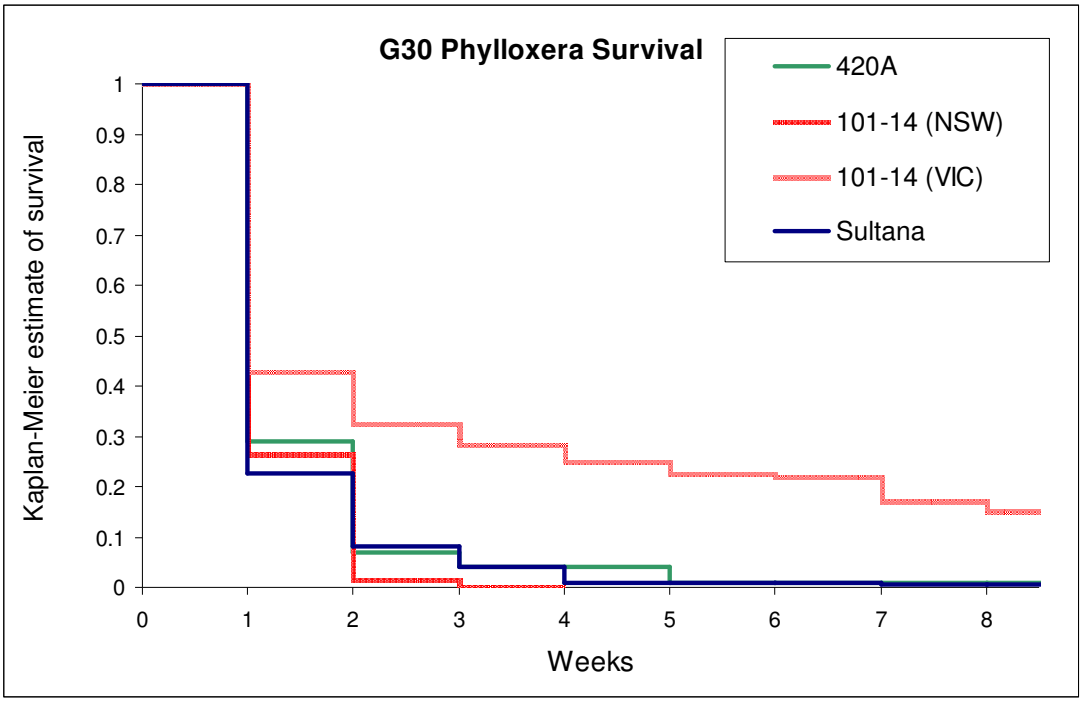


Figure 23. Excised root bioassay eleven: Comparison of phylloxera strain G30 survival on rootstocks 420A, 101-14 (NSW), 101-14 (VIC) and *V. vinifera* cv. Sultana.

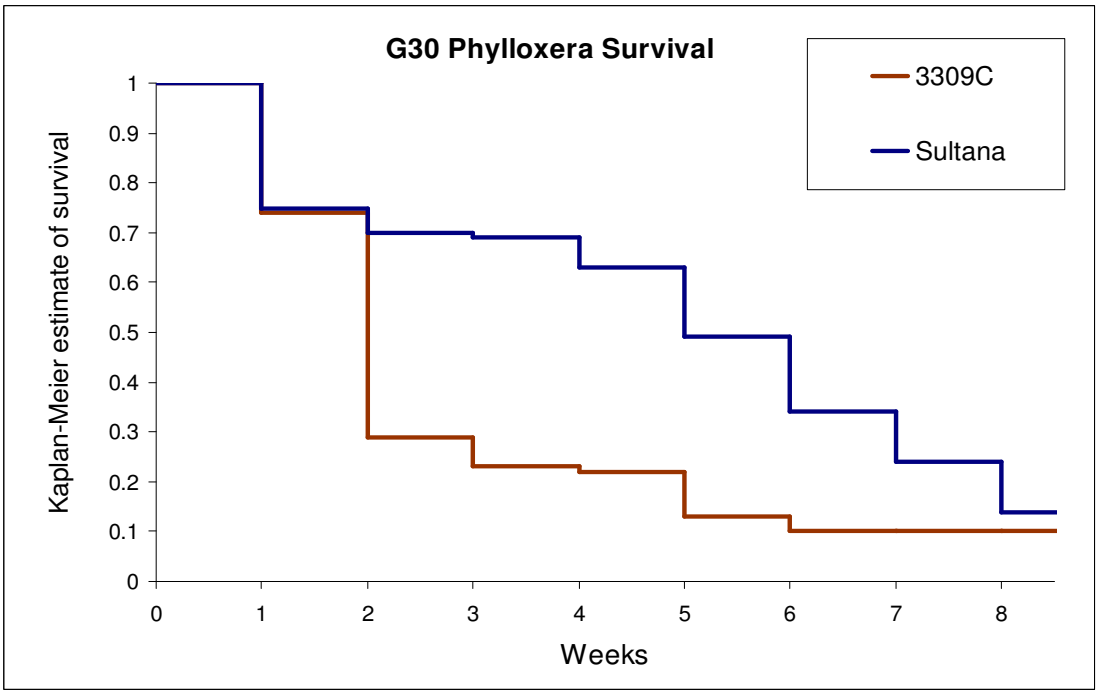


Figure 24. Excised root bioassay twelve: Comparison of phylloxera strain G30 survival on rootstocks 3309C and *V. vinifera* cv. Sultana.

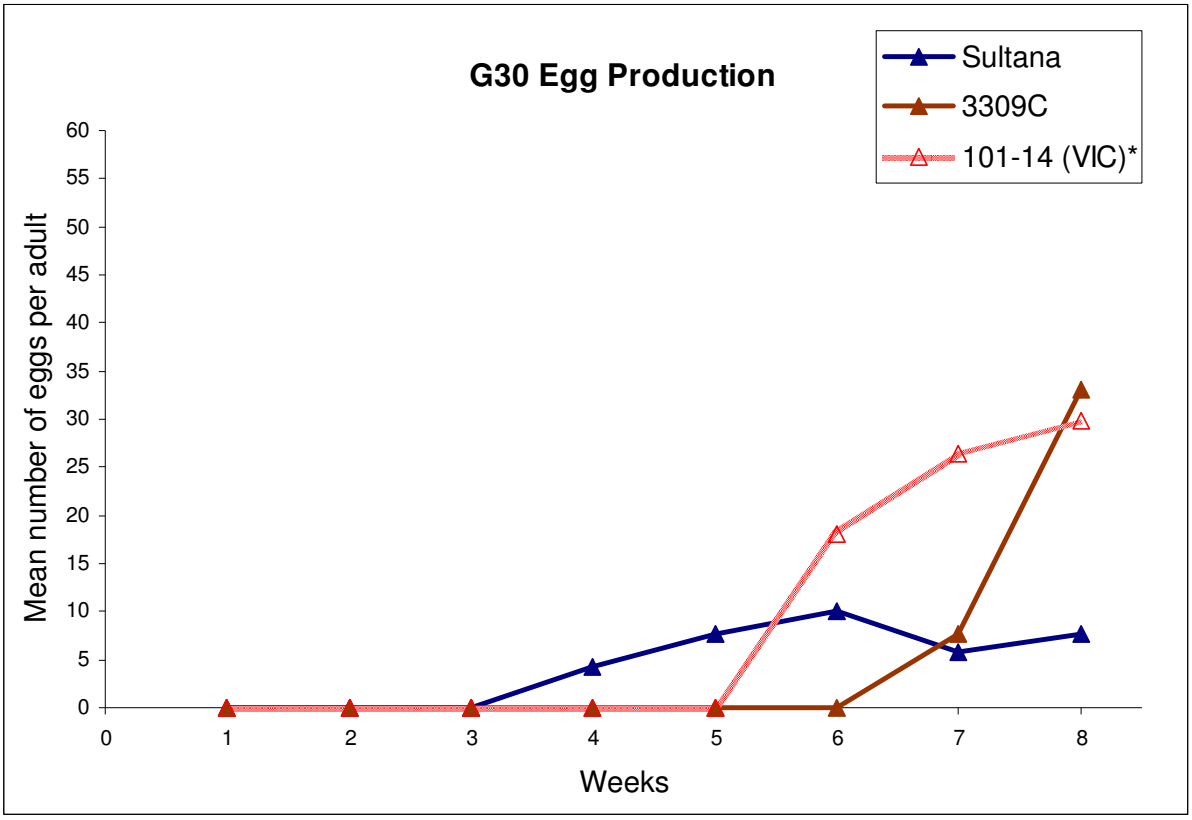


Figure 25. Excised root bioassays eleven* and twelve: Egg production of G30 phylloxera strain on 3309C, 101-14 (VIC) and *V. vinifera* cv. Sultana.

No egg production was observed on the rootstocks 420A or 101-14 (NSW) during the eight-week trial period.

Discussion

There are in Australia at least 83 known phylloxera genotypes of which six clonal lineages have been recommended for rootstock screening. Overall when comparing development and fecundity on the *V. vinifera* control the results indicate that only some phylloxera genetic strains, notably G1 and G4, are highly virulent on *V. vinifera* (Herbert *et al*., 2010). This may help to explain relative patterns of field damage and continued survival and production of ungrafted *V. vinifera* in some infested areas where low virulence strains occur. In addition virulence levels of phylloxera strains on rootstocks vary markedly. Pre-screening of rootstocks against selected phylloxera strains should be a prerequisite to enable more strategic protection of areas where particular genotypes may potentially establish.

Ratings for phylloxera 'resistance' using the excised root bioassay method described in this chapter are as follows:

- Resistant: Phylloxera does not survive > 4 weeks and no egg production
- Partial resistance: Phylloxera survives for 8 weeks and no egg production
- Tolerant: Phylloxera survives for 8 weeks at levels lower than the control and develops to adulthood and produces eggs
- Susceptible (control): Phylloxera survives for 8 weeks in higher abundance than rootstocks, and develops to adulthood and produces egg production

All four rootstocks screened in this project were generally tolerant, partially resistant or resistant (Table 4) to all tested strains of phylloxera compared with ungrafted *V. vinifera*, as shown by reduced population development on all rootstocks. However, the level of tolerance and fecundity (egg production per adult) differed between phylloxera strains and in some instances fecundity on rootstocks exceeded fecundity on the control *V. vinifera*. Of the four hybrid rootstocks tested, 420A appeared the most resistant with six phylloxera strains unable

to reach adulthood and therefore no egg production was evident. Similarly 101-14 (sourced from NSW) was relatively resistant as only a single phylloxera strain G7 could reach adulthood and produce eggs on this rootstock. In contrast, 3309C was merely tolerant to four phylloxera genetic strains all of which reached adulthood and produced eggs on this rootstock hybrid. The least resistant rootstock was 101-14 (sourced from Victoria). The rootstock 1103 Paulsen was only tested against G4 in this study and although insects could survive for up to six weeks they didn't reach adulthood.

In some instances some phylloxera strains survived for up to eight weeks on rootstocks yet did not produce eggs indicating that these rootstocks delayed life-stage development during the trial period and this may indicate partial resistance. This was particularly evident with G4 phylloxera, a highly virulent strain, with 420A, 3309C and 101-14 all showing partial resistance.

Data from this report is combined with those of Powell (2006) and Powell (2009) to provide an overall summary of *in vitro* screening of six phylloxera clonal lineages conducted to-date against 10 commercially available grapevine rootstocks (Table 5).

Table 4 Rankings of four rootstocks for resistance, tolerance and susceptibility based solely on tier one *in vitro* excised root screening under controlled conditions.

Genotype/Rootstock	***G1***	***G4***	***G7***	***G19***	***G20***	***G30***
Vitis vinifera	*S*	*S*	*S*	*S*	*S*	*S*
1103 Paulsen	-	R	-	-	-	-
420A	R	R*	R	R*	R	R*
3309C	R*	R*	T	T	T	T
101-14 (NSW)	R	R*	T	R	R	R
101-14 (VIC)	T	T	T	T	T	T

Where S= susceptible, T = tolerant, R = partially resistant (ie phylloxera survive for up to 8 weeks but do not produce eggs) and R= resistant*

Table 5 **Summary of overall rankings of eleven rootstocks for resistance, tolerance and susceptibility based solely on tier one *in vitro* excised root screening under controlled conditions.**

(Source: current chapter and also Powell 2006, 2009)

Genotype/Rootstock	*G1*	*G4*	*G7*	*G19*	*G20*	*G30*
Vitis vinifera	S	S	S	S	S	S
Ramsey	T	T	T	T	T	T
Schwarzmann	R	R	T	T	T	T
Börner	R	R	T	R	R	T
110 Richter	T	T	T	T	T	T
1103 Paulsen	T	R	R	T	R	R
140 Ruggeri	T	T	R	R	R	R
5BB Kober	R	R	T	T	T	T
420A	R	R*	R	R*	R	R*
3309C	R*	R*	T	T	T	T
101-14 (NSW)	R	R*	T	R	R	R
101-14 (VIC)	T	T	T	T	T	T

Where S= susceptible, T = tolerant, R = partial resistance (but insects survive up to eight weeks but do not produce eggs) and R = resistant; red = resistant, green = susceptible and yellow = tolerant.*

Recommendations

1. Although *in vitro* bioassays are widely used internationally and represent a useful preliminary and relatively rapid screening system, it has been reported that detached grapevine roots may produce potentially higher population abundance than when using attached roots for screening (Granett *et al*., 2001). Recent studies have shown that caution is advised when interpreting results from excised root bioassays as this type of screening protocol: (i) excludes a whole plant reaction and therefore potentially impact on host-plant defence reactions (ii) is reliant on obtaining healthy root tissue for initial screening and it is difficult to determine the physiological status of detached roots prior to screening (iii) is reliant on timing of collection of root material (i.e. vine physiology at

the time of collection may influence phylloxera-grapevine interactions) and (iv) does not consider the impact that other abiotic and biotic factors may have on the insect-host plant interactions. Therefore further screening studies are advisable under both glasshouse (Chapter 3) and field conditions (Chapter 4) to ensure rootstock recommendations are resilient in the long-term.

2. In this stuidy on some rootstocks although phylloxera developed beyond the crawler stage the population abundance was generally, but not always, lower than on *V. vinifera.* Nevertheless, some strains developed to the adult stage and produced eggs on lignified roots of rootstocks. This is not necessarily an indication of potential rootstock breakdown because root damage cannot be effectively assessed using *in vitro* bioassays alone and must be complimented with whole plant bioassays (Chapter 3).

3. Laboratory results using the excised root protocol are relatively rapid and produce results within a comparatively short time period (i.e. weeks compared to years in field trials). Whilst the *in vitro* system may not be not ideal for screening it does provide preliminary comparative screening of a range of rootstocks and allows weekly observations of phylloxera strain development. However a whole plant system under controlled conditions is physiologically closer to field conditions and other factors can be modified such as nutrition, water and soil conditions which cannot be altered in the excised root system. A rapid screening procedure in a glasshouse system is recommended as the next phase in rootstock screening as these *in planta* tests closer to the field conditions than the *in vitro* excised root bioassay system.

4. In instances where the resistance status of rootstocks to specific phylloxera genetic strains is classed as 'partial resistance' it would be strongly advised to repeat these bioassays to confirm resistance status.

5. Of the rootstocks screened in this study 3309C appears to have relatively low resistance to phylloxera than most other rootstocks tested, with four phylloxera strains able to develop to adulthood and produce eggs. Further studies on this rootstock, and also Ramsey (on which six phylloxera trains are able to develop and produce adults; Powell *et al*., 2009) should be conducted to determine the mechanism of susceptibility.

6. The results obtained when comparing 101-14 sourced from two nursery sites exemplifies why rootstock screening results from excised root bioassays need to be confirmed by repeated bioassays using different rootstock sources. This may indicate that the physiological status of the vine root system and the time of excision may influence survival and development and hence level of observed host plant resistance to different phylloxera genetic strains. It is suspected that when vines are stressed (for example heat or water stress their level of susceptibility) of phylloxera may be affected. It is also possible that rootstock clonal variation may affect phylloxera survival but this assumption would require further screening.

7. After completion of the excised root and glasshouse trials in this report Australian phylloxera genetic lineages, need to be maintained. Maintenance of separate clonal lineages of phylloxera, known to exist in Australia, under laboratory conditions is an essential prerequisite to enable future excised root bioassay screening, not only of selected commercially available rootstocks, but also newly developed hybrids.

CHAPTER 3

IN PLANTA SCREENING FOR PHYLLOXERA RESISTANCE

Output: *Information on phylloxera clones on selected rootstocks under glasshouse conditions*

Summary

Using a glasshouse rootstock screening protocol for co-cultivation of phylloxera genetic strains with potted grapevines (Powell 2006) an assessment of phylloxera development and root response under glasshouse conditions was made using six selected grape phylloxera genetic strains. These strains were contained in mesh enclosures, around the grapevine root, which allowed quantification of individual life-stages and also assessment of damage to mature lignified roots. Based on these assessments rootstocks were classed as resistant, tolerant or susceptible. As also observed using *in vitro* bioassays (Chapter 2) grape phylloxera genetic strains differed in population survival and development on both *V. vinifera* and grafted rootstock hybrids and in addition root damage levels differed between phylloxera genetic strains and rootstocks.

As shown in previous studies (Powell 2006; 2009) all six tested phylloxera strains, G1, G4, G7, G19, G20 and G30 developed on control *V. vinifera* vines, resulting in population development to the fecund (egg-laying) adult stage and necrotic tuberosity development on mature lignified roots. On *V. vinifera* relatively low phylloxera abundance were observed for strains G7, G19, G20 and G30, when compared with G1 and G4.

Three rootstocks, 3309C, 420A, 101-14 when tested against six phylloxera strains showed differences in both survival and root reaction to phylloxera feeding. 3309C was relatively tolerant with four genetic strains, surviving for at least ten weeks and developing to adulthood. 'Pseudo-tuberosities' (i.e. non-necrotic swellings on mature lignified roots) were

observed on 3309C from five phylloxera strains. The rootstock 420A was tolerant to three phylloxera strains and pseudo-tuberosities developed from three phylloxera strains. Rootstock 101-14, was the most resistant with only a single phylloxera strain, G20, developing on this rootstock and pseudo-tuberosities only observed in the presence of G19 phylloxera. 1103 Paulsen was only tested against three phylloxera strains and was resistant to all three, with no phylloxera survival after 10 weeks and no evidence of pseudo-tuberosity development. 140 Ruggeri was only tested against G20 phylloxera and was resistant to it. A summary of rootstock ratings based on glasshouse screening conducted to-date in this and previous projects (Powell, 2003; 2006) for phylloxera resistance are presented.

Methods

Plant material and growth conditions

Two trials were conducted using one-year old grapevine rootlings which were sourced from either Binjara Nurseries, Euston or Omega Nurseries, Griffith which are both located in the NSW Phylloxera Exclusion Zone (PEZ). The rootlings included five treatment bench-grafted rootstocks: 3309C, 420A, 101-14, 140 Ruggeri and 1103 Paulsen. The scion in each case was Shiraz (clone BVRC 30), including the self-grafted *V. vinifera* cv. Shiraz control to exclude grafting as a confounding factor.

All rootlings were potted at DPI-Rutherglen Centre into 20cm diameter pots with potting media (80% sterile potting mix: 20% Perlite) then kept in a shadehouse, under drip irrigation, to enable good root development before transferring them to a controlled-temperature glasshouse six months prior to treatment application (i.e. phylloxera inoculation).

Insect material

Up to six phylloxera genetic strains, G1, G4, G7, G19, G20 and G30, were used in each trial. Each genetic strain was single sourced from roots of ungrafted *V. vinifera* vines from commercial vineyards in the Nagambie (G1) and North East Victoria (G4, G7, G19, G20 and G30) PIZ's. Phylloxera strains from a single source were maintained on *V. vinifera* cv. Shiraz

excised root pieces under controlled conditions in growth cabinets (25±3°C; 12 hours L:D) using a modified method (Kingston *et al*., 2007). Prior to commencing the experiment, and at trial completion, phylloxera was sub-sampled and genotyped at CESAR, Melbourne using six microsatellite markers and rootstocks were also DNA typed (as described in Powell, 2006).

Experimental design

A randomised complete plot design was established to examine the interaction between phylloxera genetic strain and rootstock type, using a factorial design. Factors were infestation type (phylloxera genetic strain and a non-infested control) and rootstock type. Eight replicates were used per treatment in a block design to account for any variations in the environmental conditions.

Phylloxera infestation

For both glasshouse trials infestation of rootstocks with phylloxera strains occurred on separate consecutive days to prevent cross-contamination. To infest, each vine was removed from its pot and the roots cleaned of potting mix. Twenty phylloxera eggs were then placed on moistened filter paper strips. The roots of the vines were exposed, and a single, lignified root piece was selected (2.75±1.15cm diameter) and the filter paper was carefully wrapped around the root in a tube formation. A small amount of sterile soil mixture (80% potting mix, 20% Perlite™) was enclosed around the root and tube, in a mesh rectangle (8 x 10cm; 60µm pore) to form a phylloxera enclosure (Powell, 2003). The enclosure was secured at both ends and sealed with plastic cable ties and Tanglefoot™ to prevent phylloxera escaping to the rest of the root system.

The infested vine was placed in a sterile 20cm diameter pot with and addition of fresh sterile soil mixture added where required (80% potting mix, 20% Perlite™) and enclosed in a mesh bag (60µm pore size) sealed with Tanglefoot™ and cable ties to prevent cross-contamination of phylloxera genetic strains between potted vines. Two to four vines were

infested at any one time, in the laboratory, to minimise potential environmental stress to the vines.

Post infestation growth conditions

At commencement of both trials the vines were fertilised with 3.5g Osmocote™ and 500ml Thrive™ (8g of Thrive™ mixed in 4.5L water) per potted vine. Each vine was drip irrigated for two minutes daily. To prevent the vines from going into dormancy, artificial growth lights were automatically turned on each day from 6am-8pm and 1am-2am during the trial.

During the trials Gemini Tinytag Ultra™ dataloggers (Hastings Data Loggers, Port Macquarie, New South Wales) monitored the temperature and relative humidity in the glasshouse. The temperature settings were minimum 18°C and maximum 26°C 8am-9pm, min 18°C max 22°C 9pm-8am. Relative humidity was maintained at 50% using a Flipperdry 400™ de-humidifier.

Experimental harvest

After 10 weeks sequential harvesting took place, based on time period since infestation. Vines were removed from their pots and the potting mix was carefully removed from the roots to locate the phylloxera-infested root enclosure.

The exposed root system was visually checked for phylloxera and nodosities to determine if any phylloxera had 'escaped' from the enclosure. The enclosure was then removed by cutting the encapsulated root at both ends of the mesh. The enclosure was opened, rinsed with water and all material was captured in a 60 µm sieve. The infested root piece was gently scrubbed with a fine toothbrush to remove any remaining phylloxera. The washed material was then collected in tap water in a plastic vial and examined under a dissecting microscope. All phylloxera life-stages were recorded and the excised root was examined for the presence of nodosities (galls on non-lignified roots), tuberosities (necrotic swellings on lignified roots), pseudo-tuberosities (non-necrotic swellings on lignified roots) and phylloxera life-stages.

Statistical analyses

For all experiments the total abundance of phylloxera life-stages surviving after 10 weeks and the root damage (i.e. number of tuberosities or pseudo-tuberosities) was used for statistical purposes. Analyses were conducted using GenStat 12 for Windows software (Payne *et al*., 2009). Since the data was not normally distributed life-stage and tuberosity data were analysed using non-parametric methods. A Kruskal-Wallis one-way analysis of variance was used to determine the difference in rootstock resistance to each phylloxera genotype. When Kruskal-Wallis test results showed differences between the rootstocks or the genetic strains then a Mann-Whitney U (Wilcoxon rank-sum) paired test was used to compare the results between any two rootstocks.

Results

Trial 1- Phylloxera performance on V. vinifera and grafted rootstocks

Phylloxera abundance

The mean total number of phylloxera life-stages present after 10 weeks for each phylloxera clone tested on rootstocks for glasshouse trial one is shown as log data (Fig. 26). Clear differences between the development of the six phylloxera clonal lineages tested were observed.

Necrotic tuberosities developed on all control vines and 'pseudo-tuberosities' on some rootstocks (Fig. 27).

3309C

Six genetic strains, G1, G4, G7, G19, G20 and G30 were tested against 3309C. Four genetic strains, G7, G19, G20 and G30 developed on this rootstock (Fig. 26). When compared with population levels of the control *V. vinifera* only G19 showed higher levels on 3309C than the control.

Pseudo-tuberosities developed on 3309C with all phylloxera genetic strains with the exception of G1 (Fig. 27).

420A

Six genetic strains, G1, G4, G7, G19, G20 and G30 were tested against 420A. Only three genetic strains, G1, G19 and G20 developed on this rootstock (Fig. 26). When compared with population levels of the control *V. vinifera* all three genetic strains showed lower levels on 420A than the control.

Pseudo-tuberosities developed on 420A with four phylloxera genetic strains G1, G4, G7 and G30 (Fig. 27).

1103 Paulsen

Only two genetic strains, G7 and G20 were tested against 1103 Paulsen (Fig. 26). At the end of the 10 week trial neither strain survived on this rootstock. Some pseudo-tuberosities developed on 1103 Paulsen in the presence of G7 but not G20 (Fig. 27).

140 Ruggeri

Only G20 phylloxera was tested against 140 Ruggeri (Fig. 26). At the end of the 10-week trial phylloxera did not survive on this rootstock and no pseudo-tuberosities developed (Fig. 27).

Results

Trial 2- Phylloxera performance on V. vinifera and grafted rootstocks

Phylloxera abundance and root response

The mean total number of phylloxera present and tuberosity development after 10 weeks for each phylloxera clone tested on rootstocks for glasshouse trial two are shown as log data

(Figs. 28 and 29). All phylloxera strains, except G20, produced necrotic tuberosities on the control *V. vinifera* vines.

101-14

Six genetic strains, G1, G4, G7, G19, G20 and G30 were tested against 101-14. Clear differences between the performance of the six phylloxera clones on the control and 101-14 were observed. G1 had consistently higher abundance than other strains tested. Only a single phylloxera strain G20 survived on 101-14 (Fig. 28) and pseudo-tuberosities were only observed on G19 infested 101-14 plants (Fig. 29). Nodosities (data not presented) were observed with G19 and G20 only.

Nematode Observations

Although all phylloxera strains survived on *V. vinifera*, abundance on the control vines in this trial was relatively low in both trials compared with previous reports (Powell, 2003; 2006). The reasons for this are unclear however; during microscopy counting of phylloxera life-stages from root washings it was evident that high numbers of live and active nematodes were present in these samples. The origin of the nematodes was suspected to be the planting material as both potting mixture and pots were heat sterilised prior to trial commencement (as in previous studies), potting mix was supplied by the same commercial provider and in a separate study had no detrimental effects on phylloxera survival. However, grapevine rootlings used in these trials were sourced from a different nursery supplier than previously. Although identification of these nematodes (Fig. 30) was not possible they may have been entomopathogenic, as some appeared to be 'attacking' first instar grape phylloxera which may account for the lower than expected recorded phylloxera levels on the control vines.

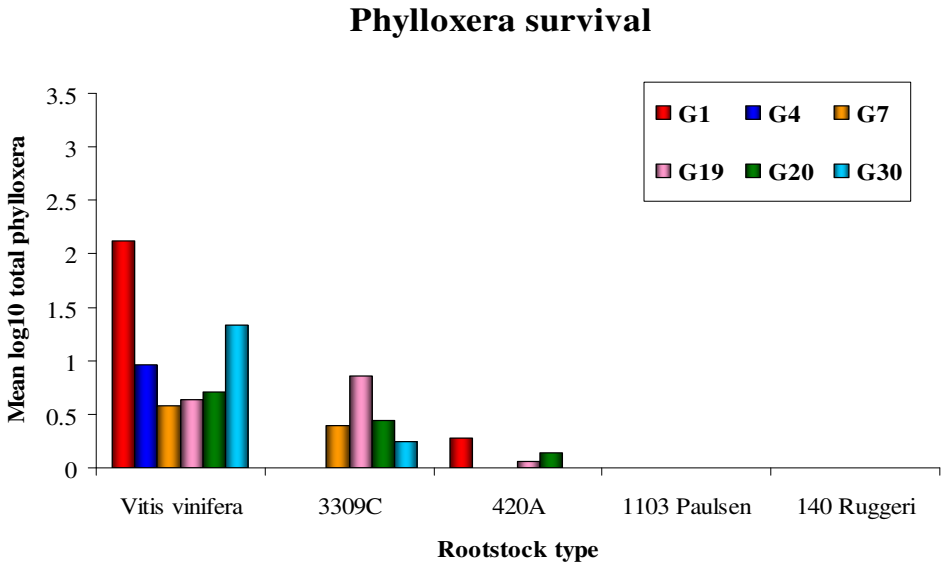


Figure 26. Total abundance of six phylloxera genetic strains, G1, G4, G7, G19, G20 and G30 phylloxera, after 10 weeks glasshouse incubation on *V. vinifera* cv. Shiraz, 3309C, 420A, 1103 Paulsen and 140 Ruggeri.

1103 Paulsen was only tested against G7 and G20 and 140 Ruggeri was only tested against G20.

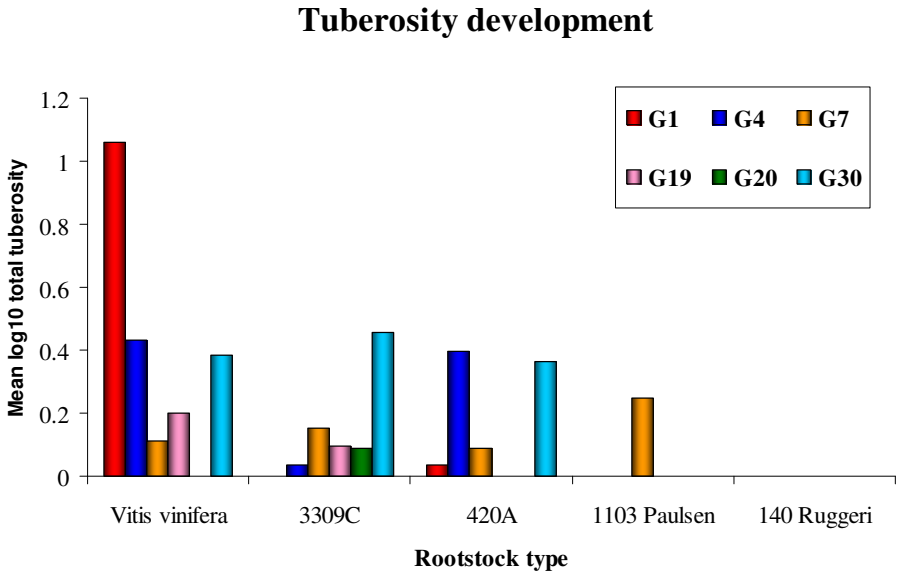


Figure 27. Total abundance of tuberosities on rootstocks caused by G1, G4, G7, G19, G20 and G30, after 10 weeks glasshouse incubation on *V. vinifera* cv. Shiraz, 3309C, 420A, 1103 Paulsen and 140 Ruggeri.

1103 Paulsen was only tested against G7 and G20 and 140 Ruggeri was only tested against G20.

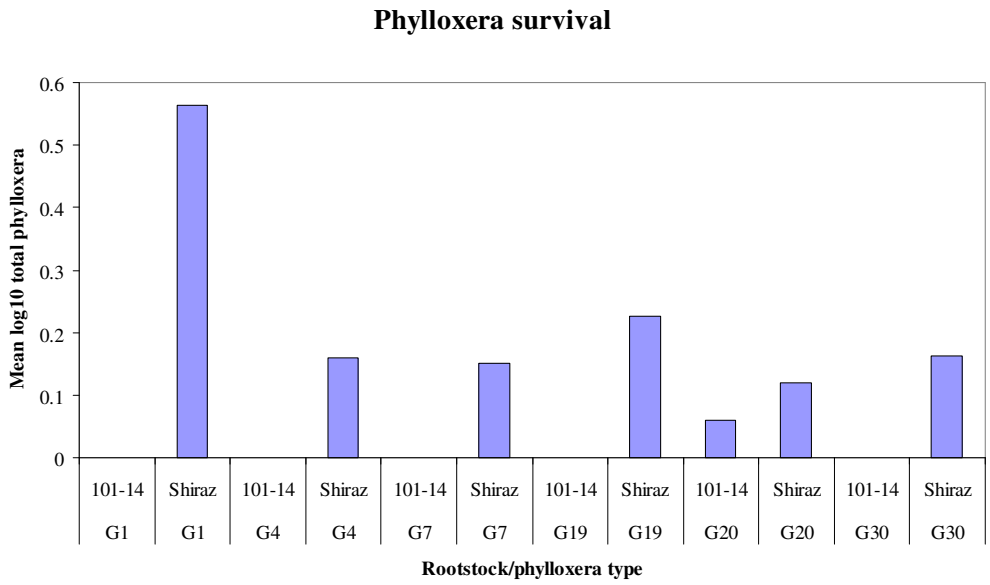


Figure 28. Total abundance of six phylloxera genetic strains, G1, G4, G7, G19, G20 and G30 phylloxera, after 10 weeks glasshouse incubation on *V. vinifera* cv. Shiraz and 101-14.

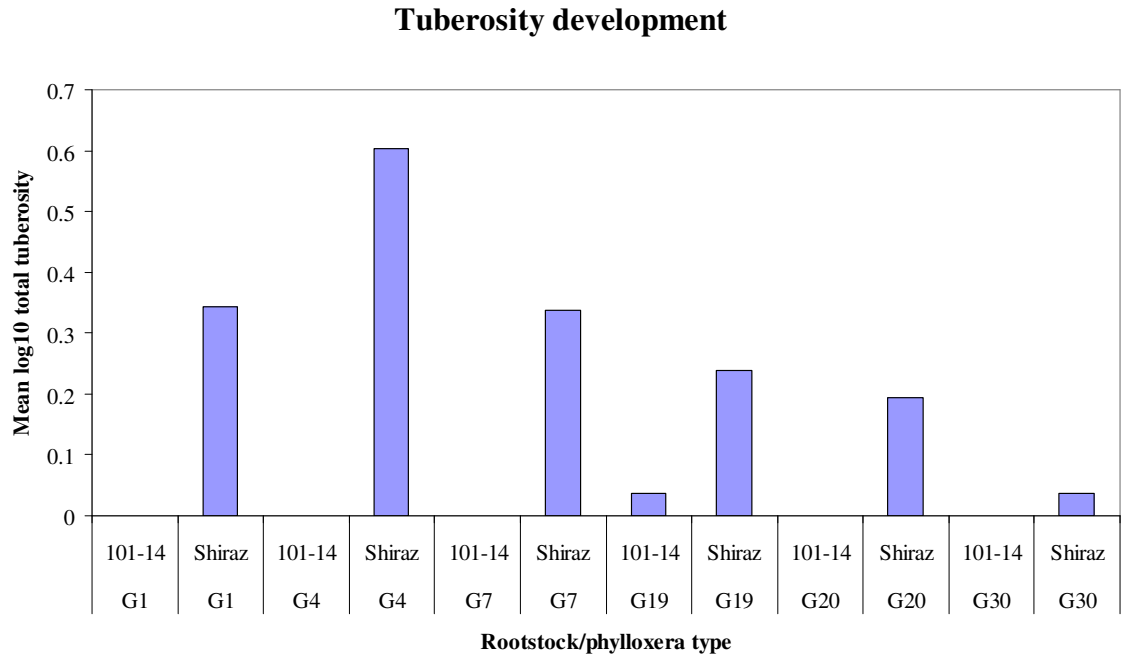
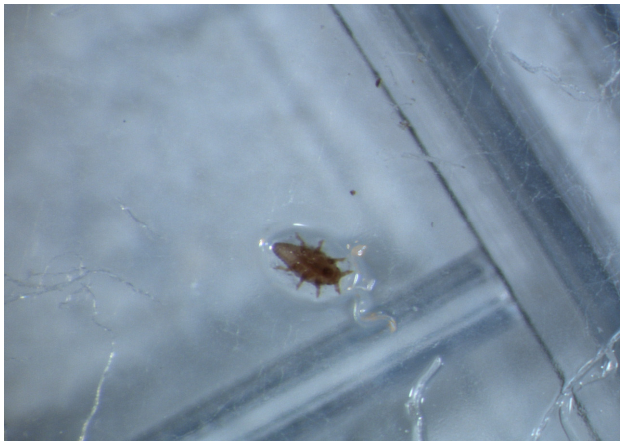


Figure 29. Total abundance of tuberosities on rootstocks caused by G1, G4, G7, G19, G20 and G30, after 10 weeks glasshouse incubation on *V. vinifera* cv. Shiraz and 101-14.

(a)



(b)

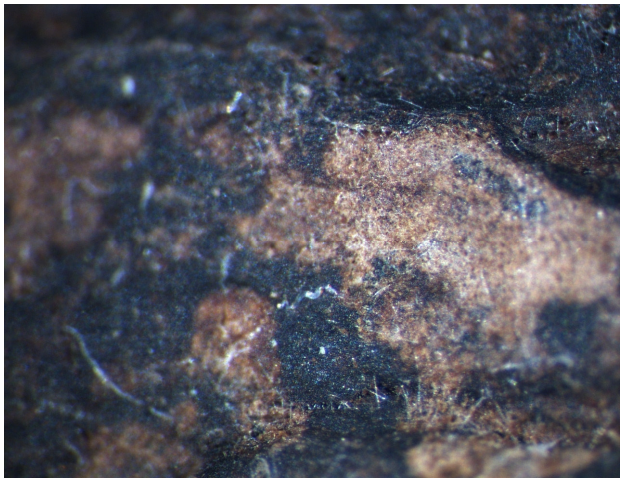


Figure 30. Unidentified nematode (a) near grapevine phylloxera first instar and (b) on *V. vinifera* lignified roots.

Discussion

Six phylloxera clonal lineages (G1, G4, G7, G19, G20 and G30) have been recommended for testing against commonly available *Vitis* hybrid rootstocks, and were tested in the two glasshouse trials presented here. *Vitis vinifera* showed susceptibility to all six phylloxera genetic strains with generally higher phylloxera abundance and more necrotic tuberosities formed on the roots than for any of the rootstocks where only non-necrotic (or pseudo-) tuberosities developed.

On the control *V. vinifera* G7, G19, G20 and G30 phylloxera showed generally relatively low abundance compared to G1 and G4. This finding provides further evidence for why G1 and G4 phylloxera 'superclones' are more abundant in phylloxera infested zones, cause more damage and are geographically relatively widespread in the field. This may also explain why G7, G19, G20 and G30 are less commonly detected. The lower abundance on *V. vinifera* of less virulent genetic strains could make these genetic strains more difficult to detect using conventional ground-surveying techniques, thereby highlighting the need for more sensitive detection methods (see Chapter 6).

Ratings for phylloxera 'resistance' using the glasshouse bioassay method described in this chapter are as follows:

- Resistant: Phylloxera does not survive 10 weeks and no egg production
- Tolerant: Phylloxera survives for 10 weeks and ± pseudo-tuberosities on mature lignified roots
- Susceptible (control): Phylloxera survives for 10 weeks and necrotic tuberosities evident on mature lignified roots

3309C was the least resistant of the rootstocks tested with four phylloxera clonal lineages (Table 6) developing to adulthood. This is a similar result to that observed in excised root bioassay trials (Chapter 2). In addition evidence of pseudo-tuberosities observed for five

phylloxera strains. The rootstock 420A tolerated three phylloxera strains and the rootstock 101-14 was the most resistant as it tolerated only one of the six phylloxera strains tested.

A table of rootstock resistance ratings (combining results from this chapter and previous published data (Powell 2006, 2009) from glasshouse studies to-date is provided (Table 7). These results indicate that selection of rootstocks with high resistance (i.e. no establishment of populations after 10 weeks under glasshouse conditions) is highly dependant on the phylloxera genetic strain tested and screening protocol utilised. For instance, Börner appears to have a broad high resistance to all six genetic strains when tested in glasshouse trials, but notably showed tolerance to two phylloxera strains in laboratory trials (Chapter 2; Table 5). In contrast Ramsey appears to have broad 'tolerance' to phylloxera, which means phylloxera can develop to the adult stage and produce eggs and the rootstock can support populations, of at least six different phylloxera genetic strains, in both *in vitro* and *in planta* bioassays. The rootstocks Schwarzmann, 5BB Kober and 3309C have similar responses to the six phylloxera strains being highly resistant to the phylloxera 'superclones', G1 and G4, but relatively tolerant to G7, G19, G20 and G30.

Table 6 Rankings of four rootstocks for resistance, tolerance and susceptibility based solely on tier two *in planta* glasshouse screening under controlled conditions.

Genotype/Rootstock	***G1***	***G4***	***G7***	***G19***	***G20***	***G30***
Vitis vinifera	*S*	*S*	*S*	*S*	*S*	*S*
1103 Paulsen	-	-	R	-	R	-
140 Ruggeri	-	-	-	-	R	-
420A	T	R	R	T	T	R
3309C	R	R	T	T	T	T
101-14	R	R	R	R	T	R

* *Where* S= susceptible, T = tolerant, R = resistant

Table 7 **Summary of glasshouse based rankings of rootstock resistance, tolerance and susceptibility based on whole plant screening under controlled environment conditions.**

(Source: current chapter and also Powell 2006, 2009)

Genetic strain/Rootstock	*G1*	*G4*	*G7*	*G19*	*G20*	*G30*
Vitis vinifera	S	S	S	S	S	S
Ramsey	T	T	T	T	T	T
Schwarzmann	R	R	T	T	T	T
Börner	R	R	R	R	R	R
110 Richter	T	T	R	R	T	R
1103 Paulsen	T	R	R	T	R	R
140 Ruggeri	R	T	R	R	R	R
5BB Kober	R	R	T	T	T	T
420A	T	R	R	T	T	R
3309C	R	R	T	T	T	T
101-14	R	R	R	R	T	R

Where ✓=- tested, x = not yet tested, S= susceptible, T = tolerant and R= resistant

As indicated in previous glasshouse trials (Powell *et al*., 2009) in addition to monitoring phylloxera development an assessment of 'tuberosity' formation may also provide a useful indicator of the level of host plant response to phylloxera infestation and may also provide a preliminary indicator of how resilient rootstock resistance may be. Tuberosity morphology varied between treatments with all *V. vinifera* screening showing necrotic tuberosities. These tuberosities provide a site for secondary fungal invasion further damaging the vine root system. Other rootstocks, depending on which phylloxera genetic strain they were infested with, developed either domed non-necrotic tuberosities (or pseudo-tuberosities) or no tuberosities indicating a different level of host plant defence response to phylloxera feeding. Although pseudo-tuberosities do not necessarily indicate any deleterious impact on vine growth there presence does indicate a differential root response which would be worthwhile monitoring under grapevine 'stressed' conditions either in the glasshouse or field to determine durability of resistance.

Recommendations

Field screening

1. Glasshouse and laboratory studies presented in Chapters 2 and 3 of this report do provide preliminary indications of relative resistance/tolerance levels of selected rootstocks against selected phylloxera genetic strains. However ultimately further rootstock and phylloxera genetic strain screening particularly under field (Chapter 4), and consequently different environmental conditions, is required in order to give Australian growers comprehensive advice on rootstock selection in particular PIZ's (where either a single phylloxera genotype or multiple phylloxera genotypes may prevail) or to protect PEZ regions, particularly from highly virulent strains, such as G1 and G4.

Rapid rootstock screening

2. Glasshouse screening has shown that phylloxera genetic strains perform differently on different rootstocks and this indicates that rootstocks may modify their feeding behaviour. One method of testing phylloxera feeding behaviour and host-plant resistance is the EPG system (Kingston *et al*., 2007) which, although recently modified for grape phylloxera, has not been tested extensively using rootstocks and does require further evaluation as a potential rapid rootstock screening method. This method if developed further could potentially provide a more rapid economical method of rootstock screening as replicates of up to eight treatments can be assessed over several hours rather than several months (or, in the case of field trials, over several seasons).

Rootstock recommendations

3. Some rootstocks tested in these trials showed high levels of resistance to some phylloxera strains, whilst others show tolerance to different strains. Further screening should be conducted using additional selected genetic strains and rootstocks to ensure that sustainable rootstock recommendations can be made. Overall the data collected supports the assumptions that the 10 rootstocks tested to-date have high to good phylloxera tolerance, although clearly the levels of tolerance do vary (Table 7) and there are some differences in ratings compared to *in vitro* studies (Chapter 2; Table 5). By comparing both *in vitro* (Chapter 2) and *in planta* bioassay data (Chapter 3; Table 7) five rootstocks (Schwarzmann, Börner, 5BB Kober, 3309C and 101-14) appear highly resistant to G1 and G4. All other rootstocks tested were tolerant to these strains (i.e. developed to the adult stage and produced eggs) with the exception of 420A which when tested against G4 phylloxera was resistant.

Host plant physiological response

4. The finding that pseudo-tuberosities (also previously reported by Powell 2006) develop, in response to phylloxera infestation, on some rootstocks under glasshouse conditions suggest a different (and possibly delayed) physiological response to infestation. These observations have not yet been confirmed under field conditions. This differential root response in tolerant rootstocks requires serious further investigation. As shown also in the previous chapter of the rootstocks screened in this study 3309C appears to have lower resistance to phylloxera than other tested rootstocks, with four phylloxera strains able to develop to adulthood and produce eggs. Further studies on this rootstock, and also 5BB Kober and Ramsey (on which six phylloxera trains are able to develop and produce adults) should be conducted to determine the mechanism of susceptibility.

Mixed genotype screening options

5. Future bioassays could incorporate mixed genetic strains of phylloxera under pot trial conditions, to determine if there is competition between strains which may influence selection of specific dominant clones. Additionally the impact of soil type, water availability and soil temperature, on phylloxera genetic strain establishment and interactions with rootstocks has rarely been considered and any potential interaction(s) could be controlled under in glasshouse conditions.

Phylloxera genotype maintenance

6. *In vitro* maintenance of separate clonal lineages of phylloxera, known to exist in Australia, under laboratory conditions is an essential prerequisite to enable future excised root bioassay screening not only of selected commercially available rootstocks but also newly developed hybrids. After completion of the excised root and glasshouse trials in these report these lineages, due to insufficient resources, will no longer be maintained and would require significant resources to re-establish (where possible).

Repeat screening and nematode identification

7. The finding in both glasshouse trials that nematodes may have reduced phylloxera levels has two important implications. Firstly because control phylloxera numbers were low means that a comparison of some rootstocks, in particular 101-14, and subsequent resistance rating based on the glasshouse trial data alone is at best speculative. It would be advisable to repeat the trial for 101-14.
8. Secondly, although the nematode species seemingly influencing phylloxera abundance could not be identified it is most likely that it had entomopathogenic properties. Entompathogenic nematodes which attack phylloxera have been investigated previously under laboratory conditions in North America (English-Loeb

et al., 1999). However to-date there have been no surveys conducted in Australian vineyards to determine if entomopathogenic nematodes are present which could potentially be used as a biocontrol option for phylloxera. This option would be worth serious consideration.

CHAPTER 4

IN SITU SCREENING FOR PHYLLOXERA RESISTANCE

Output: *Information on phylloxera clones on selected rootstocks under field conditions*

Summary

Three rootstock trials containing 6-7 commercially available rootstocks, which were originally established in phylloxera-infested vineyards in 2003 as part of project DNR 03/03 (Powell 2006), were monitored in known phylloxera-infested commercial vineyards located in Rutherglen, the King Valley and Nagambie, Victoria. In 2009 susceptible *V. vinifera* were planted into the three trial blocks, as susceptible control and marker vines for phylloxera detection (these vines could not be used for harvest assessments due to age differences between established rootstock vines and control vines). An additional trial site in the King Valley, established by a local grower, was also monitored for phylloxera presence on rootstocks, as it provided the opportunity to monitor additional rootstocks under phylloxera-infested conditions. Phylloxera was detected, on grafted rootstocks, at only two of the four trial sites monitored and was in such low numbers it would have had negligible impact on rootstock performance. However, it did highlight that even in phylloxera infested vineyards which have been replanted to resistant rootstocks there is still a risk of phylloxera transfer and quarantine breakdown. The performance of rootstocks varied between regions.

Methods

Site details

In each of the three study regions phylloxera had previously been monitored for a number of years on ungrafted vines and phylloxera genetic diversity described (Powell, 2003). In 2003 (as part of project DNR03/03) original rootstock trial plots were planted either directly in existing known infested soil, or adjacent to vine blocks known to be infested, to allow natural dispersal of phylloxera to the trial blocks.

At the Rutherglen and Nagambie sites a randomised complete block design was established. It comprised 16 blocks with eight plots of vines in each block, which were planted on 14th and 22nd October 2003 respectively. At the King Valley site a randomised complete block design was established comprising 18 blocks with eight plots in each block representing single vine treatments which were planted on 10th October 2003.

The following single vine treatments at each site were used for each plot:

1. **140 Ruggeri** (*V. berlandieri x V. rupestris*)
2. **1103 Paulsen** (*V. berlandieri x V. rupestris*)
3. **99 Richter** (*V. berlandieri x V. rupestris*)
4. **SO4** (*V. berlandieri x V. riparia*)
5. **Teleki C** (*V. berlandieri x V. riparia*)
6. **5C Teleki** (*V. berlandieri x V. riparia*)
7. **Börner (***V. riparia x V. cinerea*)

At an additional study site in the King Valley the following six additional single vine treatments were monitored:

1. **110 Richter** (*V. berlandieri x V. rupestris*)
2. **5BB Kober** (*V. berlandieri x V. riparia*)
3. **420A** (*V. berlandieri x V. riparia*)
4. **Schwarzmann** (*V. riparia x V. rupestris*)
5. **101-14** (*V. riparia x V. rupestris*)
6. **Ramsey** (*V. champini*)

Phylloxera genetic characterisation

At the King Valley field site (Site 1) G4 phylloxera was first detected in 1997 and phylloxera monitoring in nearby blocks of ungrafted *V. vinifera* have previously been reported (Powell *et al*., 2003; Herbert *et al*., 2006). An additional grower established rootstock trial site (Site 2) was also selected in the King Valley as it provided the opportunity to monitor phylloxera populations on a broader range of rootstocks.

At the Rutherglen site (Site 3) several mixed phylloxera genetic strains, including G19, had previously been reported and monitored (Powell *et al*., 2003) in an adjacent block of ungrafted *V. vinifera* cv. Cabernet Sauvignon. At the Nagambie site (Site 4) G1 phylloxera had previously been reported in several ungrafted *V. vinifera* blocks in the vineyard and monitored (Powell *et al*., 2006).

Phylloxera monitoring

For all rootstock treatments (unless otherwise stated) 10 vines were monitored from each of six to seven rootstock types at each site using emergence traps (methodology Chapter 1)

which were sampled monthly between November to May over two consecutive seasons and by a ground-based survey (methodology Chapter 5) in January 2011.

Rootstock performance

At all sites (except King Valley Site 2) to compare rootstock performance assessments of grapevine yield, quality and vigour were conducted at harvest (yield and quality) and post-harvest (pruning weights). All vines, monitored with emergence traps, were selected for both harvest and post-harvest assessments. All assessments were conducted by hand harvesting. Grape bunch number and bunch weight per vine were quantified in the field using a portable analytical balance. Samples of fifty berry weights per monitor vine were collected, weighed and transported under quarantine to the DPI-Rutherglen quarantine facility where they were crushed and the resultant juice tested for sugar content using an ATAGO™ hand held refractometer and pH using a PHM64™ pH meter.

In the autumn all monitor vines were either cane or spur pruned depending on individual growers standard practice and weighed in the field using a portable analytical balance.

Statistical analyses

All trial data was analysed using GenStat Version 12 software (Payne *et al*., 2009). Repeated Measures Analysis of Variance (ANOVA) was used for data collected over time (i.e. emergence trap data). Standard ANOVA was used for one-off events (i.e. harvest and pruning data). Where necessary data was transformed according to Log_{10} and least significant differences (LSD) were used to calculate significant differences ($p=0.05$).

Results

King Valley Site 1 - Phylloxera Monitoring

Over three consecutive seasons rootstocks were monitored for the presence of phylloxera using either emergence traps (season 2008/2009 and 2009/2010) or ground-surveying in 2011.

Control vines did not establish well and only three replicates were available for monitoring. Seven rootstock types were monitored. *No phylloxera was detected on any of the rootstock or control vines over the three monitoring seasons.*

Rootstock performance

Pruning

Pruning weights were collected in 2009 and 2011 (Table 8; Fig. 31). Data could not be collected in 2010 as the grower pruned the site prior to arrival of the research team. Overall pruning weights were significantly higher in 2011 than 2009. In 2011 pruning weights were almost double that of 2009. Significant differences in pruning weights between seasons and between rootstocks were recorded. The lowest pruning weights were recorded for Börner, 99 Richter, SO4 and 140 Ruggeri. The highest pruning weights were recorded for 5C Teleki, Teleki C and 1103 Paulsen in 2009 and 2011.

Harvest parameters

A summary of harvest data for all three seasons is shown in Table 8. All harvest parameters were significantly affected by both season and rootstock ($P<0.001$). Bunch number was influenced by season, with a significant reduction in mean bunch number and mean bunch weight across all rootstocks in 2011 due to hail damage and subsequent bunch thinning by the grower. Rootstocks with the highest bunch number varied significantly between harvests (Fig. 32). 140 Ruggeri had consistently had the lowest bunch number over all three seasons. Bunch yield and berry weight of specific rootstocks was also seasonally dependent (Figs. 33 and 34). In 2009 Teleki C produced the highest yield whilst in 2010 Börner was the highest yielding rootstock. Börner had consistently low Brix levels and high acidity across seasons (Figs. 35 and 36).

Table 8 Harvest and pruning data from a rootstock trial conducted in phylloxera infested commercial vineyard at site 1 in the King Valley, Victoria.

(a) Season 2008-2009

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean berry weight per vine (g)	Mean Brix %	Mean pH	Mean pruning weight per vine (kg)
1103 Paulsen	74	7.16	1.19	20.86	3.12	1.01
140 Ruggeri	49	4.53	1.23	21.62	3.10	0.60
5C Teleki	67	6.87	1.36	20.30	3.10	0.93
99 Richter	54	4.29	1.17	20.85	3.12	0.70
Börner	59	5.40	1.20	19.51	3.03	0.51
SO4	71	6.61	1.24	20.50	3.02	0.76
Teleki C	77	7.26	1.37	20.35	3.12	0.95

n=10, harvest date=10/3/2009, pruning date = 27/7/2009

(b) Season 2009-2010

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean berry weight per vine (g)	Mean Brix %	Mean pH
1103 Paulsen	66	4.16	1.09	23.74	3.34
140 Ruggeri	55	2.99	0.99	24.76	3.38
5C Teleki	65	4.23	1.23	23.36	3.38
99 Richter	57	3.09	1.05	23.96	3.39
Börner	68	4.56	1.17	20.96	3.18
SO4	68	4.31	1.19	22.30	3.29
Teleki C	65	4.44	1.26	22.52	3.37

n=10, harvest date=12/3/2010

(c) Season 2010-2011

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean berry weight per vine (g)	Mean Brix %	Mean pH	Mean pruning weight per vine (kg)
1103 Paulsen	13	0.43	1.35	19.80	3.22	1.52
140 Ruggeri	11	0.46	1.25	20.50	3.25	0.98
5C Teleki	17	0.81	1.47	19.46	3.25	1.58
99 Richter	15	0.52	1.29	20.30	3.20	1.11
Börner	11	0.42	1.37	19.70	3.16	0.96
SO4	14	0.55	1.41	20.24	3.20	1.31
Teleki C	13	0.54	1.56	19.76	3.24	1.53

n=10, harvest date=6/4/2011, pruning date = 23/6/2011.

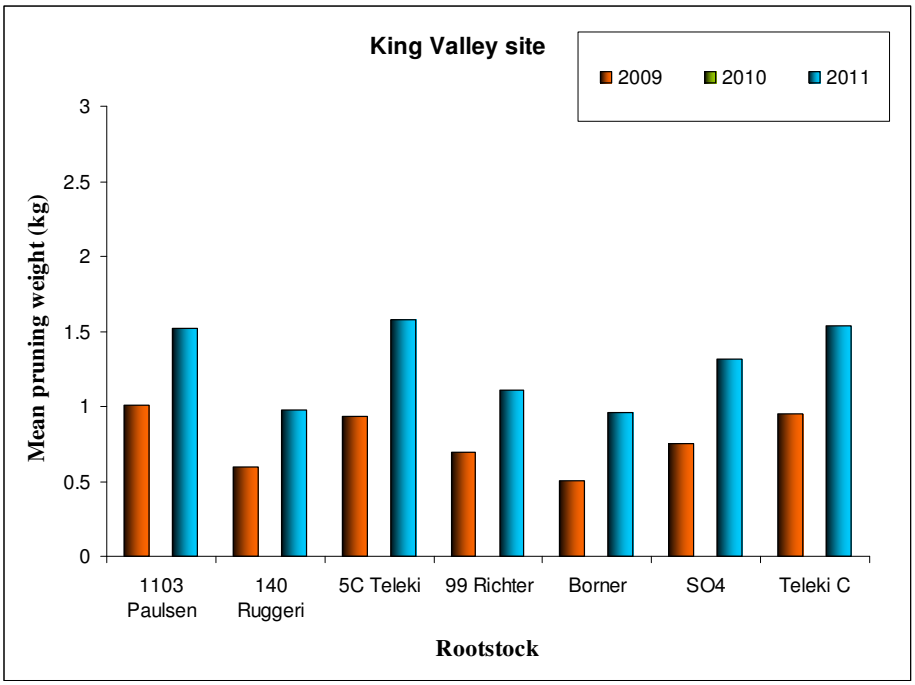


Figure 31. Mean pruning weights of seven rootstocks over two seasons at King Valley field site one.

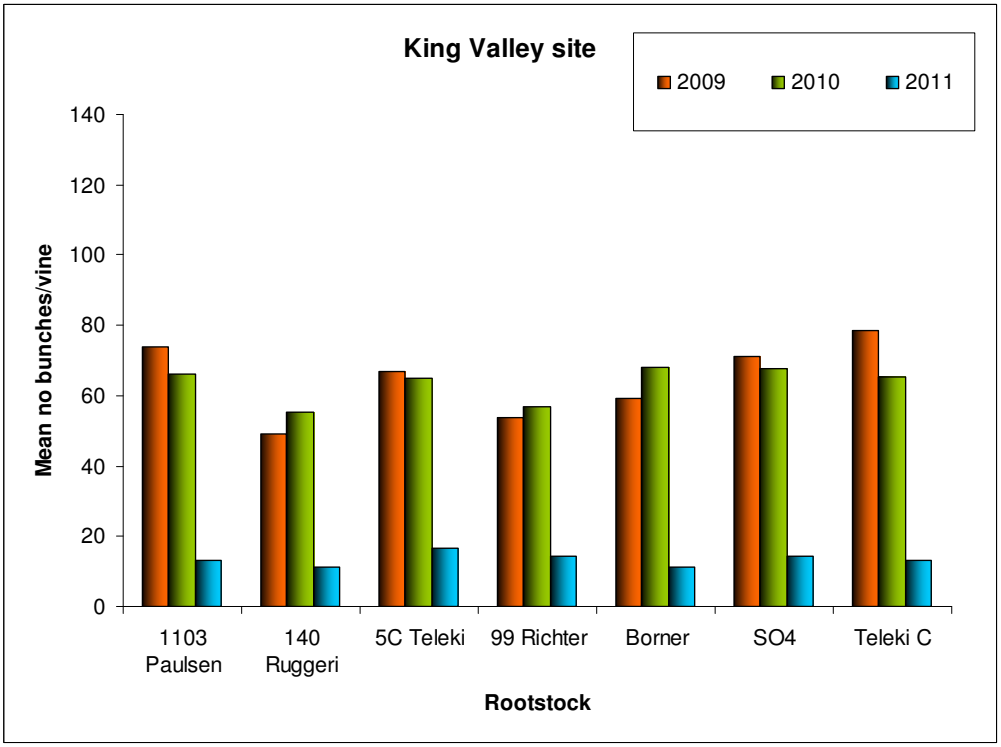


Figure 32. Mean bunch number of seven rootstocks over three seasons at King Valley field site one.

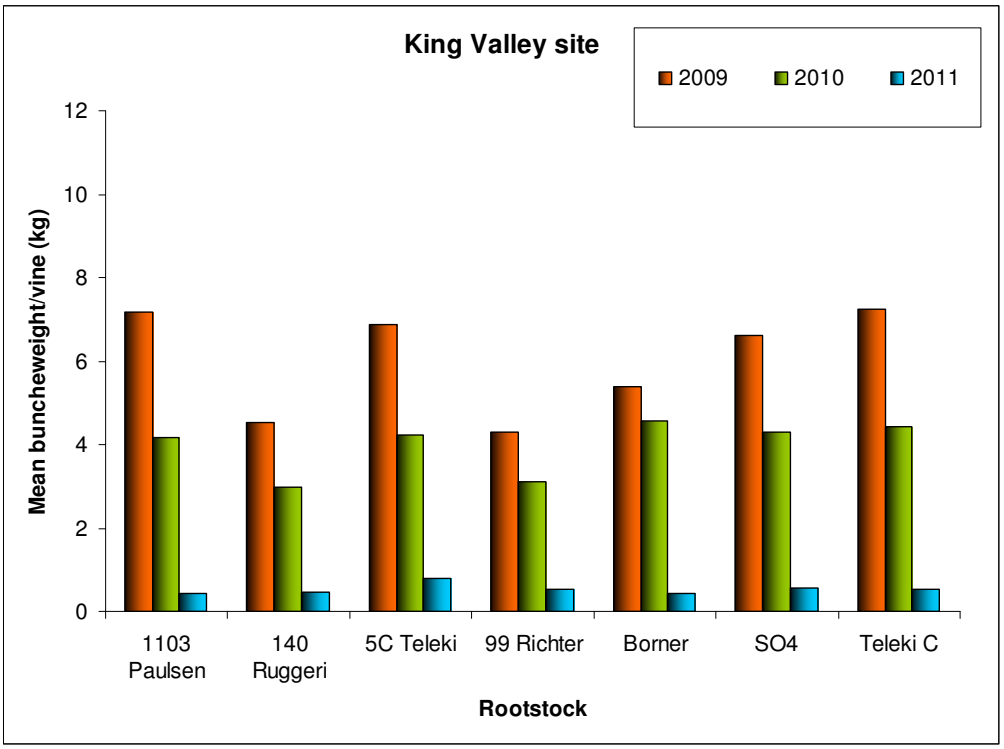


Figure 33. Mean bunch yield of seven rootstocks over three seasons at King Valley field site one.

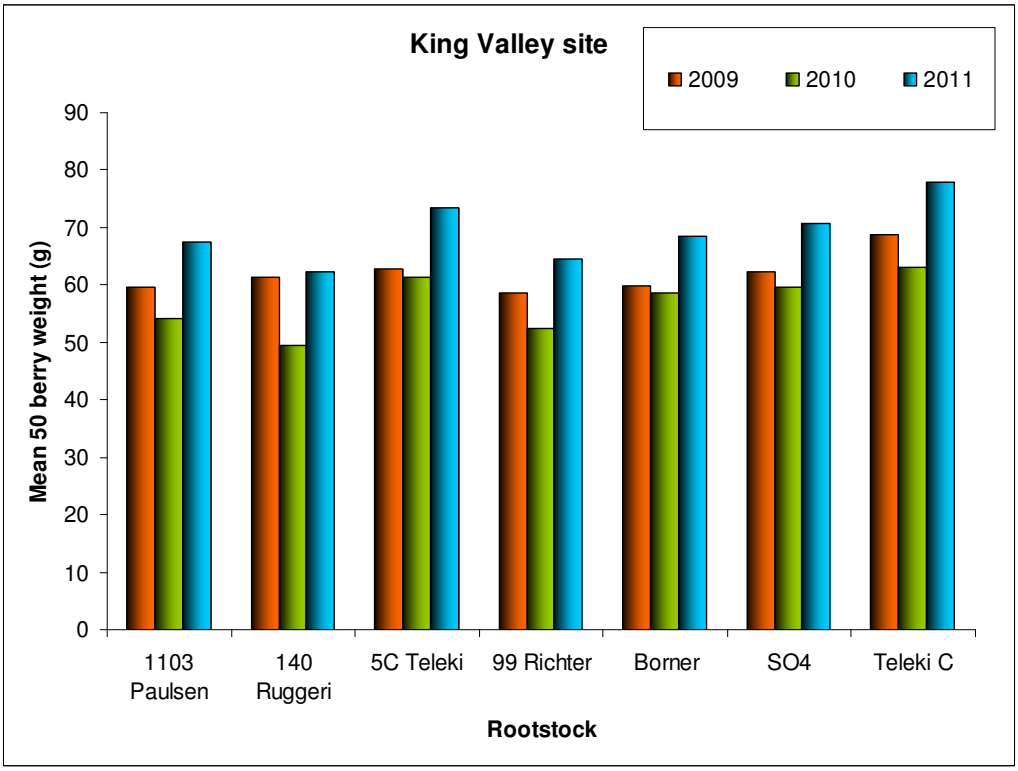


Figure 34. Mean 50 berry weight of seven rootstocks over three seasons at King Valley field site one.

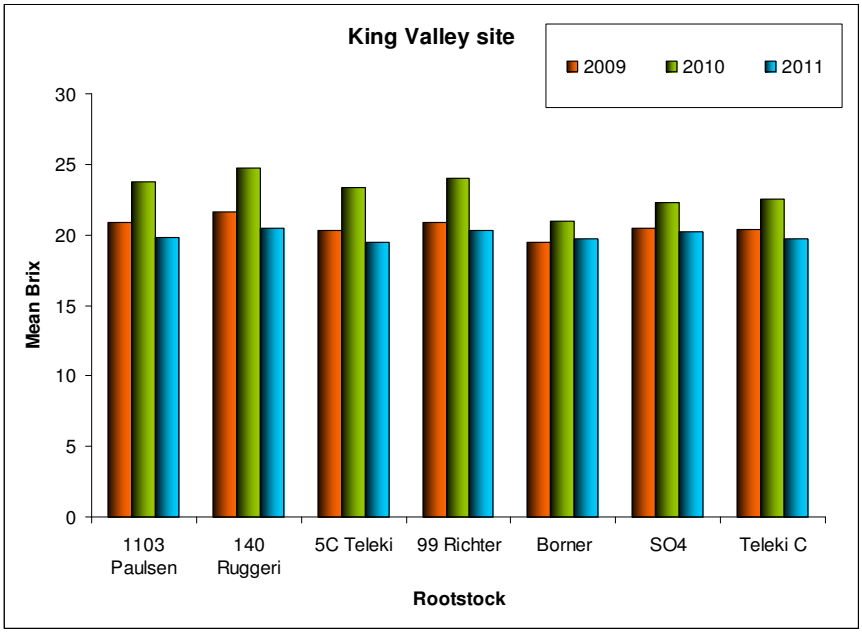


Figure 35. Mean Brix levels of seven rootstocks over three seasons at King Valley field site one.

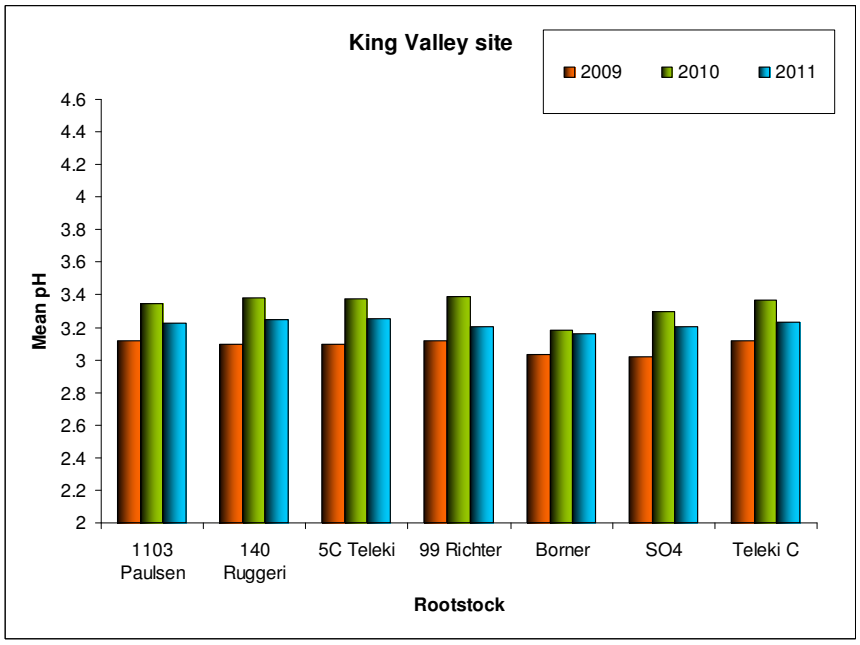


Figure 36. Mean pH levels of seven rootstocks over three seasons at King Valley field site one.

Results

King Valley site 2 - Phylloxera monitoring

Over three consecutive seasons six rootstocks were monitored for the presence of phylloxera using either emergence traps (season 2008/2009 and 2009/2010) or ground surveying in 2011. Phylloxera, genetic strain G4, was detected in low numbers on only two rootstocks 110 Richter and Ramsey (Table 9) in February 2008 and also in January 2011. In December 2009 and January 2010 phylloxera was only detected on 110 Richter.

Table 9 Relative abundance of G4 phylloxera crawlers caught over three successive seasons on rootstocks at King Valley field site two.

Rootstock/Date	**Dec 08**	**Jan 08**	**Feb 08**	**Mar 08**	**Nov 09**	**Dec 09**	**Jan 10**	**Feb 10**	**Mar 10**	**Apr 10**	**Jan 11[a]**
110 Richter	0	0	1	0	0	4	6	0	5	0	43
Ramsey	0	0	2	0	0	0	0	0	0	0	7
420A	0	0	0	0	0	0	0	0	0	0	0
101-14	0	0	0	0	0	0	0	0	0	0	0
Schwarzmann	0	0	0	0	0	0	0	0	0	0	0
5BB Kober	0	0	0	0	0	0	0	0	0	0	0
TOTAL	**0**	**0**	**3**	**0**	**0**	**4**	**6**	**0**	**5**	**0**	**50**

Where n = 10 per rootstock treatment; a = collected from root samples.

Nagambie Site 3 - Phylloxera Monitoring

Over three consecutive seasons six rootstocks (note: 99 Richter was excluded due to poor replicate survival from original 2003 plantings) were monitored for the presence of phylloxera using either emergence traps (season 2008/2009 and 2009/2010) or ground surveying in 2011. Control vines did not establish well and only 7 replicates were available for phylloxera monitoring. Despite the fact that phylloxera was known to be present in adjacent blocks no phylloxera was detected on any of the rootstock or control vines, over three consecutive growing seasons.

Rootstock performance

Pruning

Pruning weights were collected in three consecutive seasons 2009, 2010 and 2011 (Fig. 37; Table 10). Overall pruning weights were significantly higher in 2011 than 2009 and 2010. In 2011 pruning weights were two-fold more than in 2009. Significant differences in pruning weights between seasons and between rootstocks were recorded. The lowest pruning weights were recorded for Börner. Consistently highest pruning weights were recorded for 5C Teleki compared to other rootstocks monitored.

Harvest parameters

A summary of harvest data for all three seasons is shown in Table 10. All harvest parameters were significantly affected by season ($P<0.001$). Bunch number was influenced by season with a significant reduction in mean bunch number and mean bunch weight across all rootstocks in 2010. Rootstocks with the highest bunch number varied, but not significantly, between harvests (Fig. 38). 5C Teleki consistently had the lowest bunch number over all three seasons. Bunch yield and berry weight of specific rootstocks was also seasonally dependent (Figs. 39 and 40). Teleki C consistently had the highest mean berry weight. In contrast bunch yield varied between rootstocks. Börner had significantly higher acidity and relatively low Brix across all seasons (Figs. 41 and 42).

Table 10 Harvest and pruning data from a rootstock trial conducted in phylloxera infested commercial vineyard in the Nagambie, Victoria.

(a) Season 2008-2009

Rootstock	Mean total bunch number per vine	Mean bunch weight per vine (kg)	Mean berry weight per vine (g)	Mean Brix %	Mean pH	Mean pruning weight per vine (kg)
1103 Paulsen	87	4.34	1.32	19.96	3.23	0.45
140 Ruggeri	103	5.04	1.28	19.74	3.26	0.46
5C Teleki	80	4.22	1.19	19.70	3.30	0.68
Börner	94	4.28	1.25	20.18	3.21	0.40
SO4	97	4.96	1.37	20.38	3.26	0.55
Teleki C	100	4.99	1.41	20.00	3.33	0.60

n=10, harvest date=12/3/2009; pruning date = 30/7/2009.

(b) Season 2009-2010

Rootstock	Mean total bunch number per vine	Mean bunch weight per vine (kg)	Mean berry weight per vine (g)	Mean Brix %	Mean pH	Mean pruning weight per vine (kg)
1103 Paulsen	83	5.85	1.10	18.90	3.39	0.63
140 Ruggeri	88	6.63	0.97	18.19	3.34	0.55
5C Teleki	71	5.44	0.96	18.33	3.36	0.88
Börner	85	6.42	1.09	18.10	3.27	0.58
SO4	84	7.48	1.18	18.26	3,26	0.58
Teleki C	83	6.92	1.20	18.30	3.39	0.64

n=10, harvest date=5/3/2010; pruning date = 24/6/2010.

(c) Season 2010-2011

Rootstock	Mean total bunch number per vine	Mean bunch weight per vine (kg)	Mean berry weight per vine (g)	Mean Brix %	Mean pH	Mean pruning weight per vine (kg)
1103 Paulsen	91	8.11	1.51	20.40	3.20	1.78
140 Ruggeri	99	8.52	1.58	20.34	3.18	1.63
5C Teleki	77	8.60	1.53	19.82	3.19	1.88
Börner	98	9.21	1.55	19.80	3.13	1.05
SO4	89	9.63	1.71	19.68	3.15	1.70
Teleki C	97	10.94	1.63	19.42	3.20	1.63

n=10, harvest date=22/3/2011; pruning date = 27/6/2011.

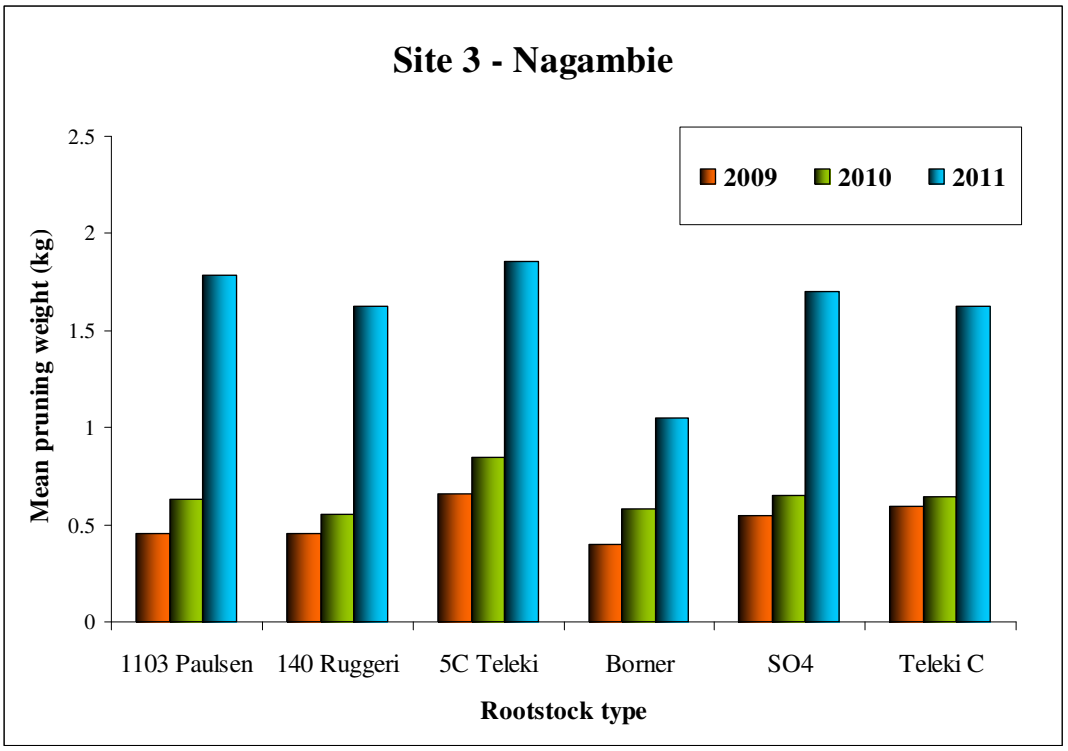


Figure 37. Mean pruning weights of six rootstock types over three seasons at Nagambie field site three.

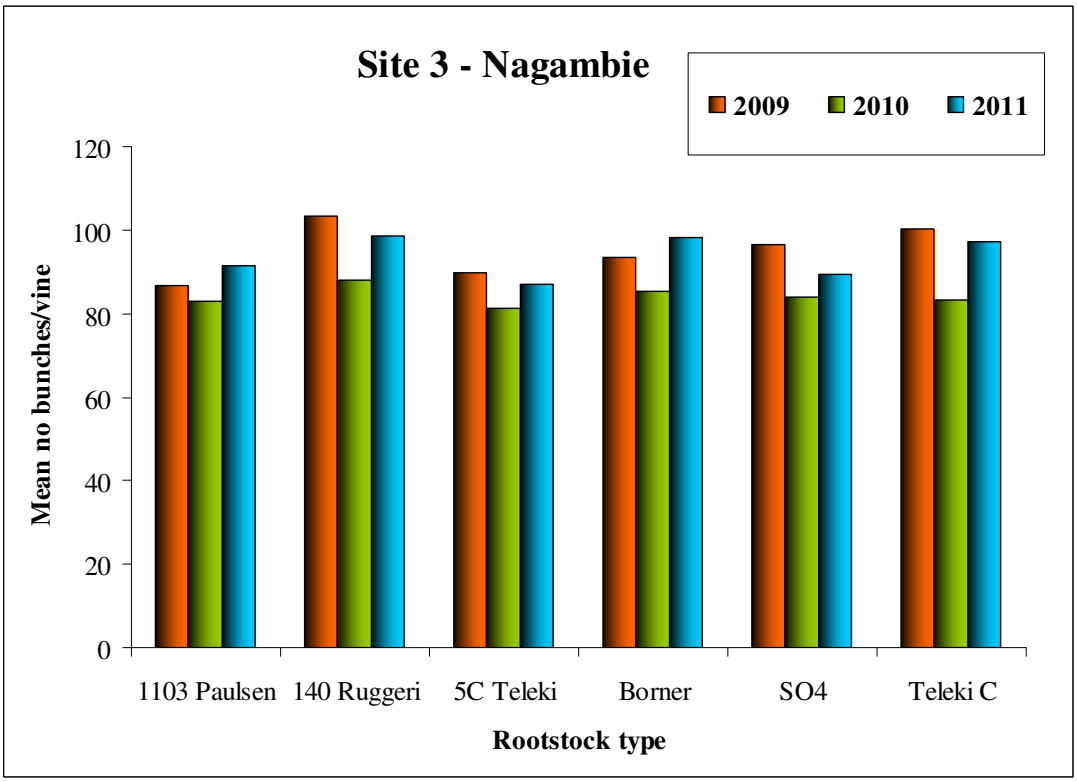


Figure 38. Mean bunch number of six rootstocks over three seasons at Nagambie field site three.

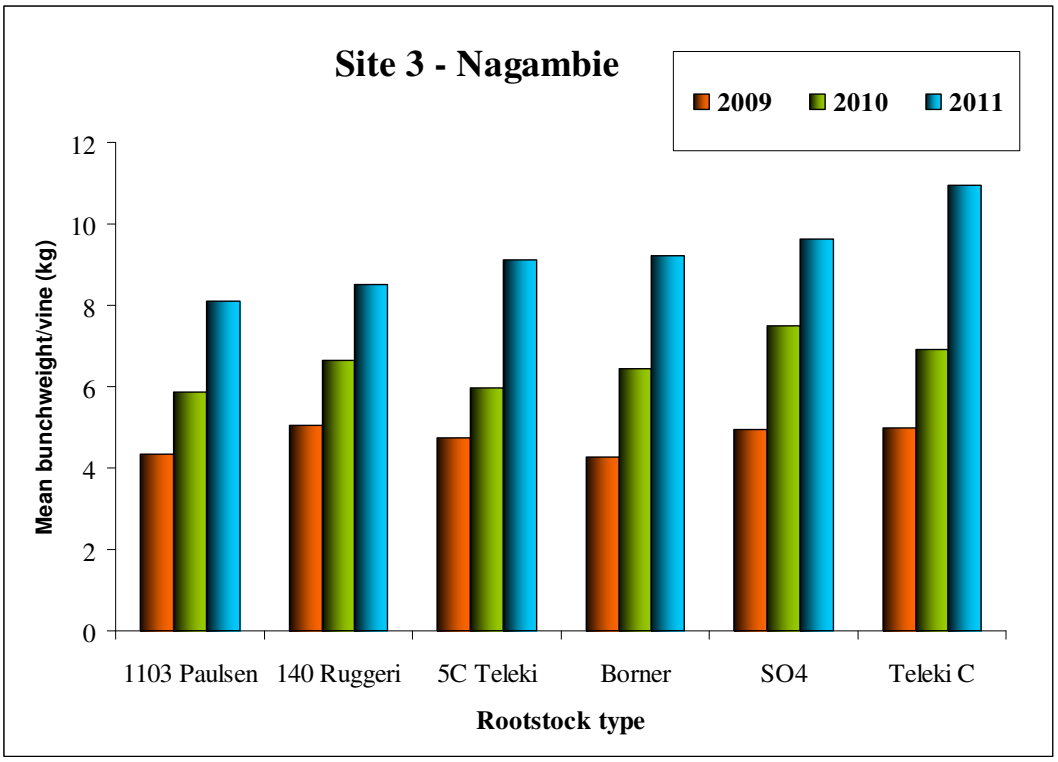


Figure 39. Mean bunch yield of six rootstocks over three seasons at Nagambie field site three.

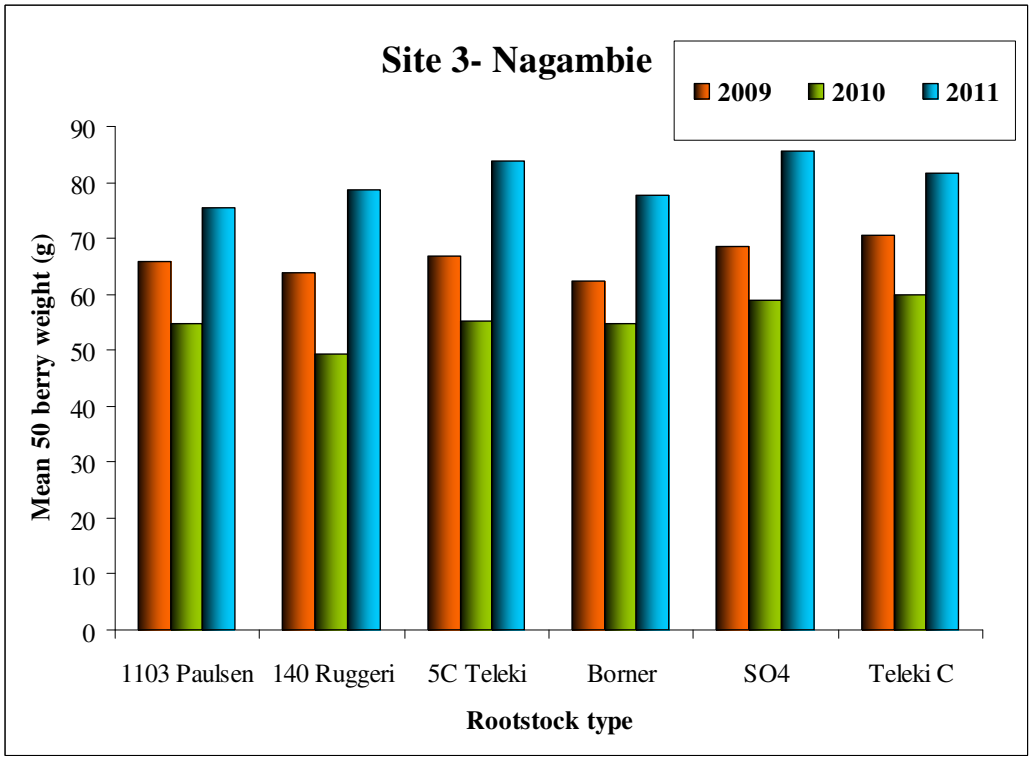


Figure 40. Mean 50 berry weight of six rootstocks over three seasons at Nagambie field site three.

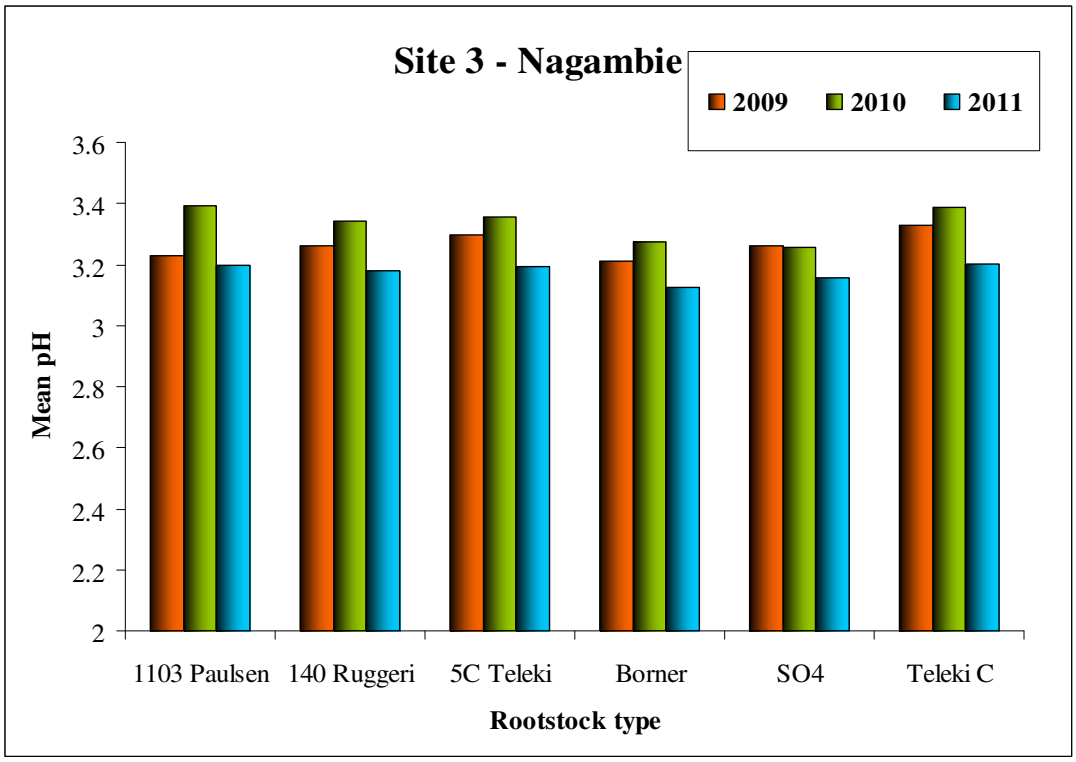


Figure 41. Mean Brix levels of six rootstocks over three seasons at Nagambie field site three.

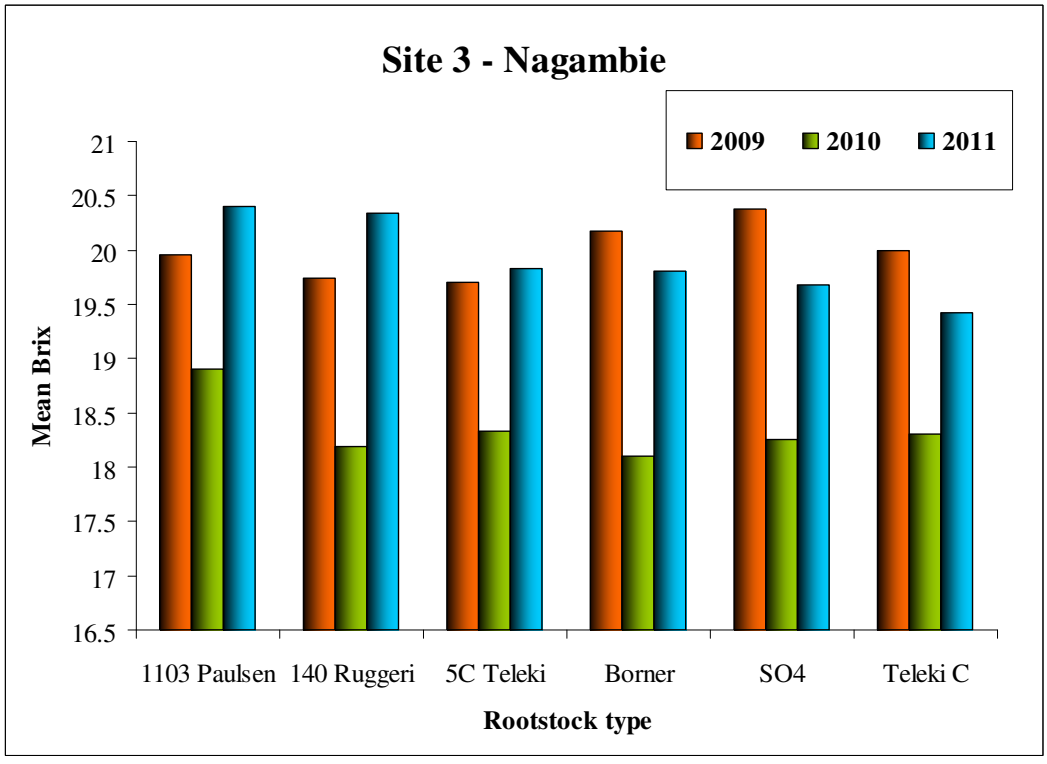


Figure 42. Mean pH levels of six rootstocks over three seasons at Nagambie field site three.

Rutherglen Site 4 - Phylloxera Monitoring

Over three consecutive seasons six rootstocks were monitored for the presence of phylloxera using either emergence traps (season 2008/2009 and 2009/2010) or ground surveying in 2011 at the Rutherglen site. All treatments and control vines were monitored from each of seven rootstock types using emergence traps which were sampled monthly between November to April over two consecutive seasons and by emergence traps in January 2011 only. Due to relatively poor establishment (from original 2003 plantings) only 5 replicates of each treatment and control were available for phylloxera monitoring.

Phylloxera genetic strain G19 was detected in low numbers on the rootstocks 1103 Paulsen and SO4 in season 2009-2010 (Table 11). However, G19 phylloxera was most prevalent on Börner and was detected in five monthly sampling periods in the 2008-09 season, and in three monthly sampling points in the 2009-10 season.

Table 11 Relative abundance of G19 phylloxera crawlers caught over three successive seasons on rootstocks at Rutherglen field site four.

Rootstock/Date	**Nov 08**	**Dec 08**	**Jan 08**	**Feb 08**	**Mar 08**	**April 08**	**Nov 09**	**Dec 09**	**Jan 10**	**Feb 10**	**Mar 10**	**Apr 10**	**Jan 11**
140 Ruggeri	0	0	0	0	0	0	0	0	0	0	0	0	0
1103 Paulsen	0	0	0	0	0	0	2	0	0	0	0	0	0
SO4	0	0	0	0	0	0	0	0	1	0	0	0	0
Teleki C	0	0	0	0	0	0	0	0	0	0	0	0	0
Börner	2	62	16	0	11	8	0	0	4	1	5	0	0
99 Richter	0	0	0	0	0	0	0	0	0	0	0	0	0
5C Teleki	0	0	0	0	0	0	0	0	0	0	0	0	0
V. vinifera	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	**2**	**63**	**16**	0	**11**	**8**	**2**	**0**	**5**	**1**	**5**	**0**	**0**

Where n=5 per rootstock and control treatment

Rootstock performance

Pruning

Pruning weights were collected in two consecutive seasons 2010 and 2011 (Fig. 43; Table 12). The lowest pruning weights were recorded for Börner. Consistently highest pruning weights were recorded for 5C Teleki compared to other rootstocks monitored. The highest pruning weights were recorded for 140 Ruggeri.

Harvest parameters

A summary of harvest data for all three seasons is shown in Table 12. All harvest parameters were significantly affected by season ($P<0.001$) and rootstock (with the exception of Brix). Bunch number was influenced by season with higher mean bunch number across all rootstocks in 2011. Rootstocks with the highest bunch number varied between harvests (Fig. 44). Bunch yield and berry weight of specific rootstocks was also seasonally dependent (Figs 45 and 46). Brix levels and pH showed no consistent rootstock effect (Figs. 47 and 48).

Table 12 Harvest and pruning data from a rootstock trial conducted in phylloxera infested commercial vineyard in the Rutherglen, Victoria.

(a) Season 2008-2009

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean berry weight per vine (g)	Mean Brix %	Mean pH
1103 Paulsen	47	5.49	0.80	22.56	3.82
140 Ruggeri	75	8.54	0.95	22.40	3.78
5C Teleki	73	8.65	0.92	22.95	3.75
99 Richter	70	5.65	0.80	22.70	3.84
Börner	76	5.34	0.87	22.70	3.68
SO4	66	7.55	0.87	23.20	3.77
Teleki C	74	8.08	0.86	23.85	3.87

n=5, harvest date=27/2/2009

(b) Season 2009-2010

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean berry weight per vine (g)	Mean Brix %	Mean pH	Mean pruning weight per vine (kg)
1103 Paulsen	42	5.86	0.99	23.55	3.99	1.52
140 Ruggeri	77	8.68	1.08	24.60	4.05	2.67
5C Teleki	75	10.84	1.17	23.80	3.92	1.77
99 Richter	77	7.58	0.98	24.01	4.04	1.94
Börner	57	6.97	0.91	24.56	3.87	1.37
SO4	60	8.96	1.05	24.36	3.88	1.99
Teleki C	58	7.72	1.01	24.36	4.05	1.78

n=5, harvest date=1/4/2010; pruning date=29/6/2010

(c) Season 2010-2011

Rootstock	Mean total bunch number per vine	Mean total bunch weight per vine (kg)	Mean berry weight per vine (g)	Mean Brix %	Mean pH	Mean pruning weight per vine (kg)
1103 Paulsen	68	4.80	0.86	18.35	3.63	1.78
140 Ruggeri	119	7.66	1.01	20.40	3.67	2.46
5C Teleki	99	6.81	0.89	18.56	3.60	1.58
99 Richter	101	5.91	1.02	20.64	3.75	2.25
Börner	96	5.37	0.91	19.72	3.54	1.46
SO4	94	5.96	0.94	19.40	3.63	1.80
Teleki C	102	5.53	0.90	19.16	3.69	1.77

n=5, harvest date=25/3/2009; pruning date=20/6/2011

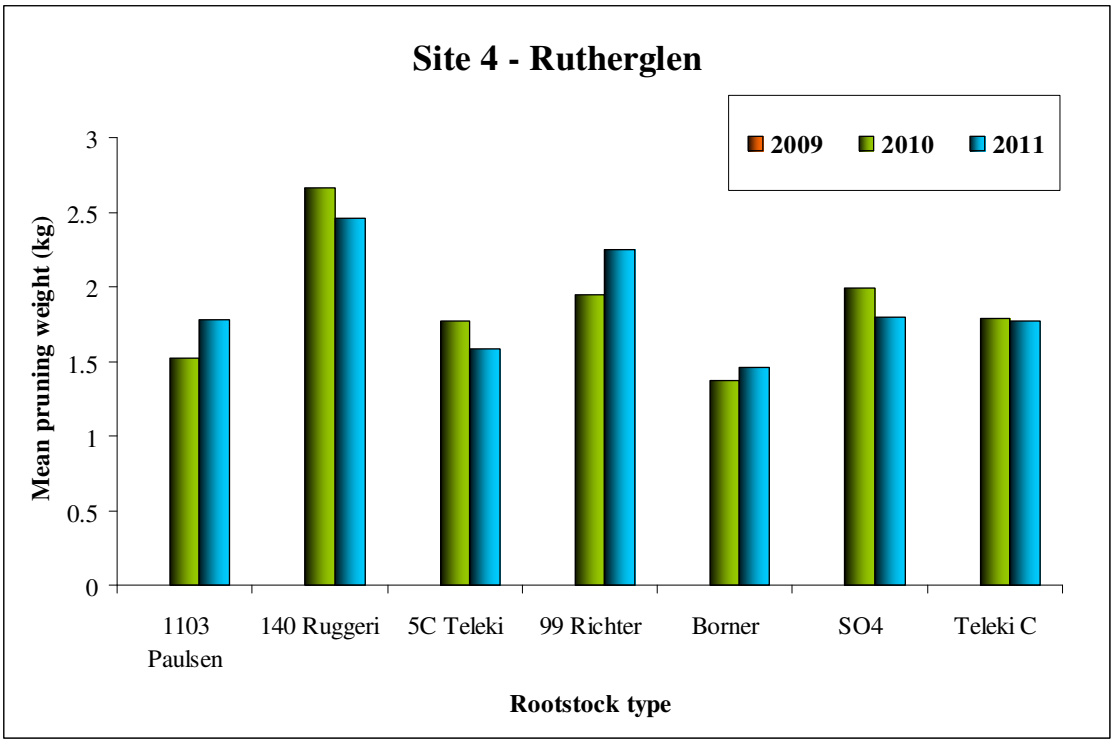


Figure 43. Mean pruning weights of seven rootstocks over two seasons at Rutherglen field site four.

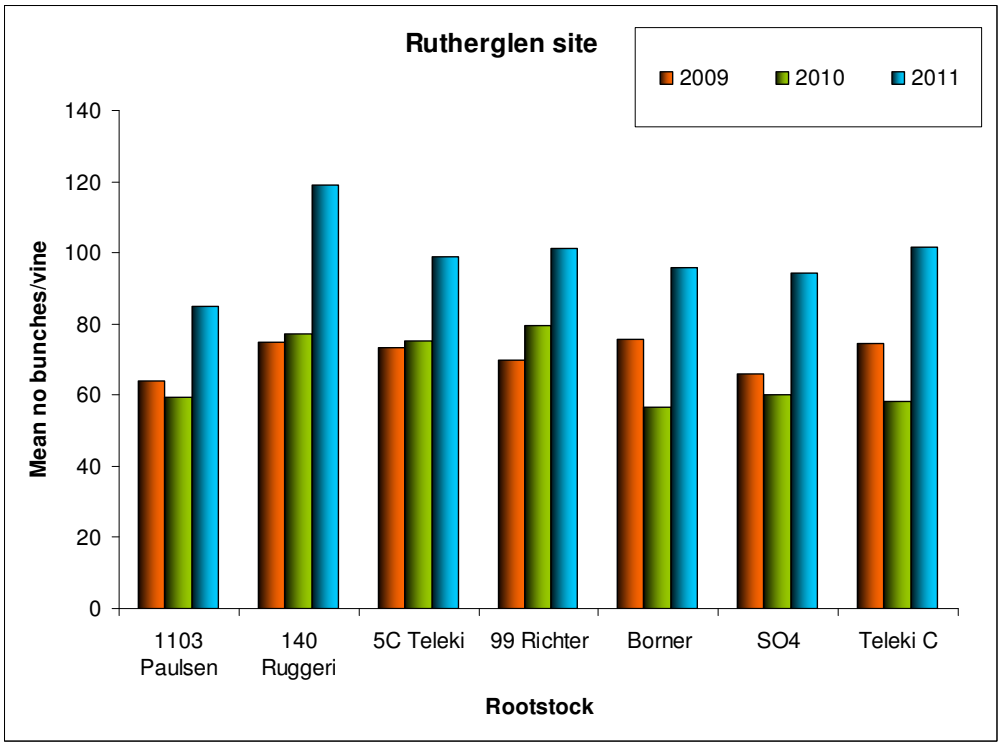


Figure 44. Mean bunch number of seven rootstocks over three seasons at Rutherglen field site four.

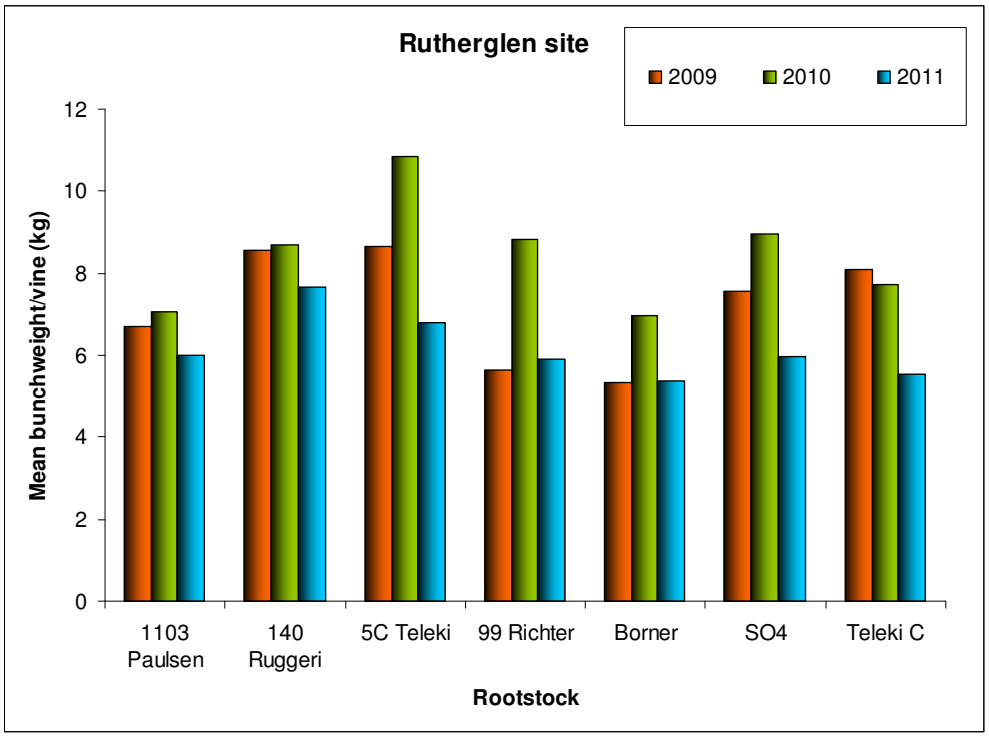


Figure 45. Mean bunch yield of seven rootstocks over three seasons at Rutherglen field site four.

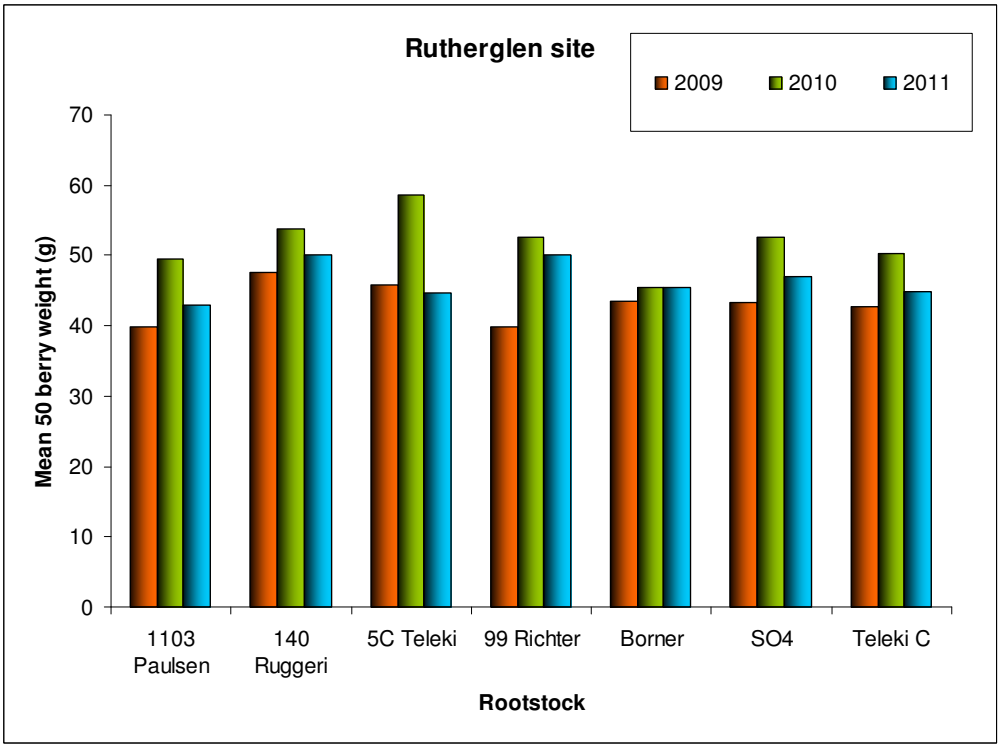


Figure 46. Mean 50 berry weight of seven rootstocks over three seasons at Rutherglen field site four.

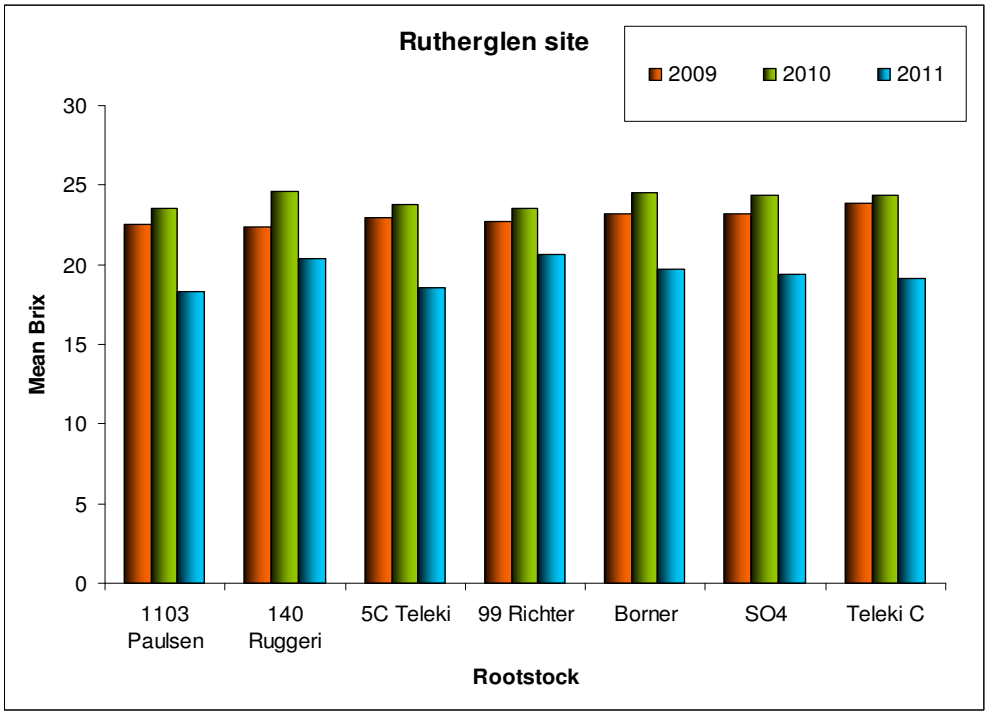


Figure 47. Mean Brix levels of seven rootstocks over three seasons at Rutherglen field site four.

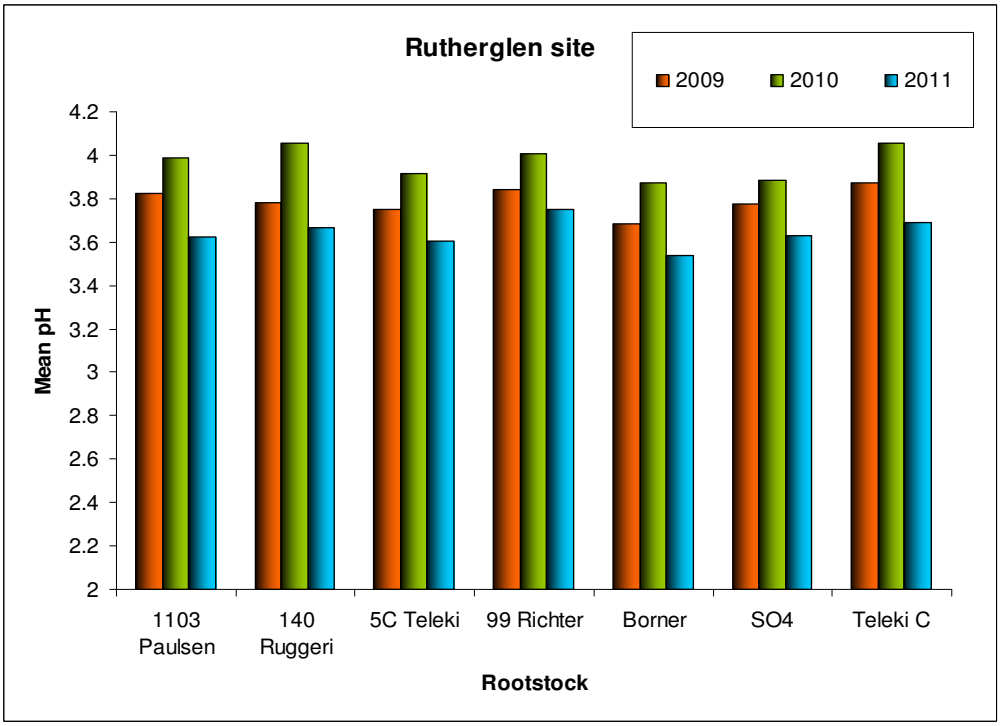


Figure 48. Mean pH levels of seven rootstocks over three seasons at Rutherglen field site four.

Discussion

Having four rootstock trials in three different phylloxera-infested regions offered the unique opportunity for (i) a comparative field screening of commercially available rootstocks under different soil and climatic conditions, (ii) an assessment of interactions at a field level between rootstocks and phylloxera genetic strains and (iii) an assessment of the risk of phylloxera transfer from infested vineyards replanted to resistant rootstocks. These trials were established in 2003 and unfortunately monitoring between 2004-2008, due to funding constraints, could not be implemented until 2008 which was five years post-planting. As baseline data on phylloxera abundance levels and spatiotemporal distribution was therefore not available it is unclear whether the rootstocks actually reduced the phylloxera levels.

However at two of the sites phylloxera was detected, albeit at low levels, on some rootstocks. At one King Valley site G4 was detected on Ramsey and 110 Richter. This is the first time G4 has been recorded in the field on these two rootstocks. Although no visual above ground damage was apparent the presence of G4 on these two rootstocks highlights a quarantine risk for grafted vineyards where G4 phylloxera is present. G4 has previously been shown to survive to adulthood and produce eggs on Ramsey and 110 Richter, under both laboratory and glasshouse conditions (Chapters 2 and 3)).

At the Rutherglen field site G19 phylloxera was detected on 1103 Paulsen, which has also been observed under laboratory and glasshouse conditions, and SO4. However G19 was more abundant on Börner. This finding, highlights that Börner may not be completely immune (as suggested in some literature) to phylloxera under field conditions even though laboratory and glasshouse trials indicate it appears to be one of the more resistant rootstocks screened in Australia to-date.

Based on the availability and location four established rootstock field sites, only three phylloxera genetic strains could be monitored in the field. In addition some rootstocks of interest (as recommended by the NPTRG in 2008) had not been planted at the sites in 2003 and therefore could not be assessed under field conditions. Despite these limitations some

valuable data was obtained (Table 13) showing that some rootstocks were resistant (i.e. no phylloxera present over three consecutive seasons) and others tolerant (i.e. phylloxera present either on roots or in emergence traps). In a previous study (Powell, 2006) on established rootstock trials located in the Nagambie and North East Victoria PIZ's additional phylloxera genotypes have been monitored and when this data is combined (with that presented in this Chapter) it is apparent where the information gaps exist (Table 14) for the 10 rootstocks of interest. For example no rootstock trials have been conducted in vineyards where G20 phylloxera predominates. This genotype is known to exist in the North East PIZ and was responsible for the phylloxera detection in the Buckland Valley in 2003. Other notable gaps are the paucity of field data on phylloxera genotype interactions with cool-climate preferred rootstocks such as 420A, 101-14 and 3309C which have only been tested in the King Valley where only G4 phylloxera predominates.

Table 13 Rankings of thirteen rootstocks for resistance or tolerance based solely on tier three *in situ* field screening under commercial vineyard conditions.

Genotype/Rootstock	G1	G4	G19
1103 Paulsen	**R**	**R**	**T**
101-14	**-**	**R**	**-**
140 Ruggeri	**R**	**R**	**R**
Ramsey	**-**	**T**	**-**
Schwarzmann	**-**	**R**	**-**
Börner	**R**	**R**	**T**
110 Richter	**-**	**T**	**-**
5BB Kober	**-**	**R**	**-**
420A	**-**	**R**	**-**
SO4	**R**	**R**	**T**
Teleki C	**R**	**R**	**R**
99 Richter	**-**	**R**	**R**
5C Teleki	**R**	**R**	**R**

Where - = no available data, T = tolerant, R = resistant

Table 14 **Combined summary of field based rankings of rootstock resistance, tolerance and susceptibility based on *in situ* field screening of ten rootstocks in commercial vineyard trials**
(*includes data from Powell 2006-shown in italics*)

Genotype/Rootstock	*G1*	*G4*	*G7*	*G19*	*G20*	*G30*
Vitis vinifera	S	S	S	S	S	S
Ramsey	*R*	T	-	-	-	-
Schwarzmann	*T*	R	*T*	-	-	*R*
Börner	R	R	-	T	-	-
110 Richter	*T*	T	*T*	-	-	*R*
1103 Paulsen	R	R	-	T	-	-
140 Ruggeri	R	R	R	-	-	-
5BB Kober	*T*	R	*T*	-	-	*R*
420A	-	R	-	-	-	*R*
3309C	-	-	-	-	-	-
101-14	-	R	-	-	-	-

Where: - = not yet tested, S= susceptible, T = tolerant and R= resistant

Recommendations

1. Although relatively extensive rootstock screening has been conducted for phylloxera resistance under controlled conditions (Chapters 2 and 3) significant information gaps still exist relating to screening for phylloxera resistance under field conditions. Of the eleven commercially available rootstock types highlighted in this report under field conditions G4 phylloxera has been tested against nine, G1 against seven, G7 against four, G30 against three, G19 against two and G20 against none.

2. G4 is a highly virulent strain particularly in cool climate conditions (Chapter 1) but no data is available on its resistance to 3309C under field conditions. This rootstock has been tested in laboratory and glasshouse conditions where it appears to be only tolerant to some phylloxera genotypes and it therefore needs to be tested under

phylloxera-infested field conditions to fully determine its suitability for planting to protect against phylloxera.

3. Although G1 is the most geographically widespread and highly virulent phylloxera strain identified to-date in Australia we have no data on its resistance to 420A, 3309C or 101-14 under field conditions. This highlights a significant information gap and these rootstocks should therefore be tested under G1 phylloxera-infested field conditions.

4. Very limited field data exist on rootstock resistance for genotypes G7, G19, G20 and G30, which although not currently widespread geographically have the potential to become more significant in the future and further field trials, in areas where these genotypes persist are recommended.

5. These information gaps highlighted above should be clearly addressed by either more field-based rootstock trials, genomic studies on phylloxera or comparative electrophysiological studies (Kingston *et al*., 2007) to ensure robust rootstock recommendations are provided to the Australian viticulture industry.

CHAPTER 5

IMPROVED QUARANTINE PROTOCOLS

Output: *Scientific validation of National Phylloxera Management Protocols - White Juice*

Summary

Grape phylloxera is a significant insect pest of grapevines. Dispersive stages of phylloxera, particularly first instars, can be inadvertently transferred from infested vineyards on several potential 'vectors' including post-harvest grape products (Deretic *et al*., 2003).

Phylloxera population dynamic studies conducted worldwide in infested vineyards (King and Buchanan 1986; Porten and Huber 2003; Powell *et al*., 2003) have identified that the most dispersive life-stage of phylloxera is the first instar nymph commonly referred to as the 'crawler', which are present in the vine canopy, on both foliage and fruit, during the growing season (Powell *et al*., 2000). Phylloxera crawlers have been detected on mechanical harvesters (King and Buchanan 1986) and they can subsequently survive some post-harvest grape handling processes, such as transport in grape bins, crushing, destemming and pressing (Deretic *et al*., 2003). This indicates the possibility of transferring live dispersive stages of phylloxera on grape products from infested vineyards.

In order to move juice from a PIZ or PRZ (Phylloxera Risk Zone) to a PEZ the existing disinfestation regulations (Procedure D in National Phylloxera Management Protocol; NVHSC 2009) are that juice must be either fermented for at least 72 hours or filtered through a 50 micron filter. The fermentation component of this protocol is based on experimental data originally developed using red juice. White juice is processed differently to red juice, and has a number of different physiochemical properties to red juice and undergoes a range of treatments prior to filtering (Rankine 1995). For example, tartaric acid may be added to ensure pH is within a range of 3.0-3.4. At various stages in the white wine-making process sulphur dioxide is generally added, as either potassium or sodium metabisulphite. At maturity

Baumė is usually between 10º and 12.5º depending on variety. Cool temperatures are generally used for white grapes with fermentation occurring at 10-16ºC or lower, clarification occurring below 15ºC and cold stabilisation occurring at -4 to +2ºC. The impact of white juice, pH, Baumé, sulphur dioxide and cold temperature on phylloxera mortality has either never before been examined or only conducted under limited conditions.

Through a series of laboratory-based experiments the impact of white juice, pH, sugar concentration, sulphur dioxide content and cold temperature on grape phylloxera crawler mortality where examined. In this study we tested a highly virulent phylloxera genetic strain G4 in a range of treatment combinations to find the optimal conditions over the shortest-time period to achieve 100% phylloxera mortality during white juice processing. Based on a range of normal winemaking procedures, and discussion with NPTRG, the variables and ranges selected were:

Duration: 1-8 days

Temperature: 2-10ºC

pH: between 3.0 and 3.7

Baumé: between 11º and 14º

Sulphur dioxide: at 20ppm

Of the treatments tested cold temperatures had the most significant impact on survival, particularly 2ºC which resulted in 100% mortality in white juice at 2 days. Temperature impacts on phylloxera survival and development have primarily focused previously on warmer optimal temperatures for development (Granett and Timper 1987; Turley *et al*., 1996; Fisher and Albrecht 2003; Makee 2004) and also lethal upper temperature limits (Korosi *et al*., 2011b) and little is known of phylloxera's ability to withstand temperatures ≤10º C.

In the absence of a National Phylloxera Technical Reference Group (which was disbanded in 2010) the results of this work will require endorsement by an equivalent

National Biosecurity committee before they can be incorporated in the National Phylloxera Management Protocols.

Materials and methods

Insect maintenance

The G4 phylloxera genetic strain was single sourced from an infested vineyard in the King Valley, Northeast Victoria PIZ G4 phylloxera was established as a stock culture using an excised grapevine root system (Kingston *et al*., 2007) and genotyped using mitochondrial DNA markers (Umina *et al*., 2007).

All phylloxera life-stages were carefully manipulated using a fine sable hair paintbrush to prevent damage. Prior to treatment freshly oviposited phylloxera eggs were carefully removed from the excised *V. vinifera* cv. Sultana root pieces. They were then placed in damp filter paper lined Petri dishes, sealed with Parafilm™ and covered with aluminium foil, to exclude light. The eggs were then incubated at 25±2°C for 5-7 days in a constant temperature room. On hatching, newly emerged phylloxera first instars were removed and used in all risk analysis trials.

Bioassay design

The impact of treatment on phylloxera first instars (crawlers) was assessed using immersion chambers. Round plastic, screw-top desiccators (13cm[h] x9.5cm[d]) were used as a 'bioassay treatment chamber'. Within each of two bioassay treatment chambers 400 ml of treatment or control solution was added. Ten newly-emerged phylloxera first instars were placed on a 1cm^2 moist filter paper strip. Each strip was placed into a modified plastic vial (5.5cm [h]; 2.5cm [d]). The top and base of each vial was cut out and replaced with a 25µm pore stainless steel mesh that prevented phylloxera escape while still allowing treatment or control solution to enter the vial. To restrict vial buoyancy, and ensure vials were fully immersed in solution throughout each trial period, they were weighed down with a Petri-dish lid and each single

vial filled with solution. Once vials had been fully immersed the chamber lid was screwed on and placed under constant temperature conditions. Temperatures ranged from 2-10ºC. Phylloxera first instar survival, at 24h intervals, was assessed in the range of 1-10 days. During all trials Gemini Tinytag Ultra™ dataloggers (Hastings Data Loggers, Port Macquarie, New South Wales) monitored the temperature in each controlled environment chamber. Crawler survival was assessed using a low powered binocular microscope.

White grapes

Chardonnay grapes were hand harvested, in two consecutive vintages 2009 (pH 4.4 and Brix 24.4) and 2010 (pH 4.2 and Brix 22.4), crushed, pressed and the resultant juice stored at -20ºC prior to testing.

White juice and pH

To test the affect of pH on phylloxera first instar survival laboratory trials were conducted under quarantine conditions at the DPI Victoria-Rutherglen Centre. Single sourced G4 phylloxera was laboratory reared on excised roots (as described earlier) and used in this trial. Treatment pH of either 3.0 or 3.7 was obtained by addition of tartaric acid. Chardonnay juice was adjusted by dilution in Ultra pure water (UPW) where necessary to maintain a Baumé of 11.7º. Survival in the acidic white juice was compared to survival in UPW (adjusted to acidic pH using tartaric acid) or UPW with a pH of 6.7. Ten replicates, of ten crawlers per replicate, of each treatment and control were used. Phylloxera first instar survival was assessed 5ºC for all treatments and controls.

White juice and Baumé

To test the affect of Baumé on phylloxera first instar survival, laboratory trials were conducted under quarantine conditions at the DPI Victoria-Rutherglen Centre. Single sourced G4 phylloxera laboratory reared on excised roots (as described earlier) were used in this trial. A standardised juice of pH 3.4 was obtained by addition of tartaric acid. UPW was used as an

additional control (pH 6.7). Chardonnay juice was adjusted by dilution in UPW where necessary to maintain Baumé of either 11.1º or 14.1º.

A control mixture of glucose (47.04g)/fructose (57.16g) was also used which was diluted in distilled water to obtain a Baumé of 11.0º or 14.0º. The ratio of glucose/fructose was based on a ratio for Chardonnay mixture (Kliewer 1967). The pH of the sugar solution ranged from 5.7 to 7.1. To maintain temperature all experiments were conducted in a controlled temperature room. Ten replicates, of ten crawlers per replicate, of each treatment and control were used. Phylloxera first instar survival was assessed at 5ºC.

White juice and cold temperature

To test the affect of cold temperature and white juice on phylloxera first instar survival, laboratory trials were conducted under quarantine conditions at the DPI Victoria-Rutherglen Centre. Single sourced G4 phylloxera was laboratory reared on excised roots (as described earlier) was used in this trial. Phylloxera first instar survival was assessed at three temperatures 2, 5 and 10ºC. Ten replicates, of ten crawlers per replicate, of each treatment and control were used.

Chardonnay juice pH was adjusted with tartaric acid to a standard pH of 3.4. Sugar content was standardised by dilution in UPW where necessary to maintain Baumé of 11.1º. A control of UPW was also used and pH adjusted to 3.4 using tartaric acid. To maintain temperature experiments were conducted in controlled environment chambers.

A control mixture of glucose (47.04g)/fructose (57.16g) was also used which was diluted in distilled water to obtain a Baumé of 11.0º. To maintain temperature experiments were conducted in controlled temperature rooms.

White juice and sulphur dioxide

To test the affect of sulphur dioxide on phylloxera first instar survival laboratory trials were conducted under quarantine conditions at the DPI Victoria-Rutherglen Centre. Single sourced G4 phylloxera was laboratory reared on excised roots (as described earlier) were used in this

trial. Phylloxera first instar survival was assessed at two temperatures 2ºC and 10ºC. Chardonnay juice was adjusted by dilution in UPW where necessary to maintain Baumé of 11.5º. A standardised juice pH of 3.4 was obtained by addition of tartaric acid. A sulphur dioxide concentration of 20ppm was obtained by the addition of potassium metabisulphite at a rate of 33.3mg/L to each treatment and control. Ten replicates, of ten crawlers per replicate, of each treatment and control were used.

Results

White juice and pH

Phylloxera survival at 5ºC in Chardonnay juice adjusted to pH 3.0 and 3.7 with Baumé standardised at 11.7º was compared with survival in two water controls: one adjusted to acidic pH 3.0 using tartaric acid and another unadjusted with variable pH (Fig. 49). In the adjusted ph water control insects survived for at least 10 days and at least eight days in the control water with variable pH. This is similar to previously reported data on phylloxera survival in water at a neutral pH (Korosi *et al*., 2009).

The combination of juice and acidic pH did however significantly reduced survival by 2-3 days indicating that some other characteristic of white juice, other than pH alone, reduces phylloxera survival. Phylloxera survived for up to 5 and 7 days, at pH 3.7 and 3.0 respectively, when combined with white juice at 5ºC compared with survival of 8-10 days on the controls (Fig. 49).

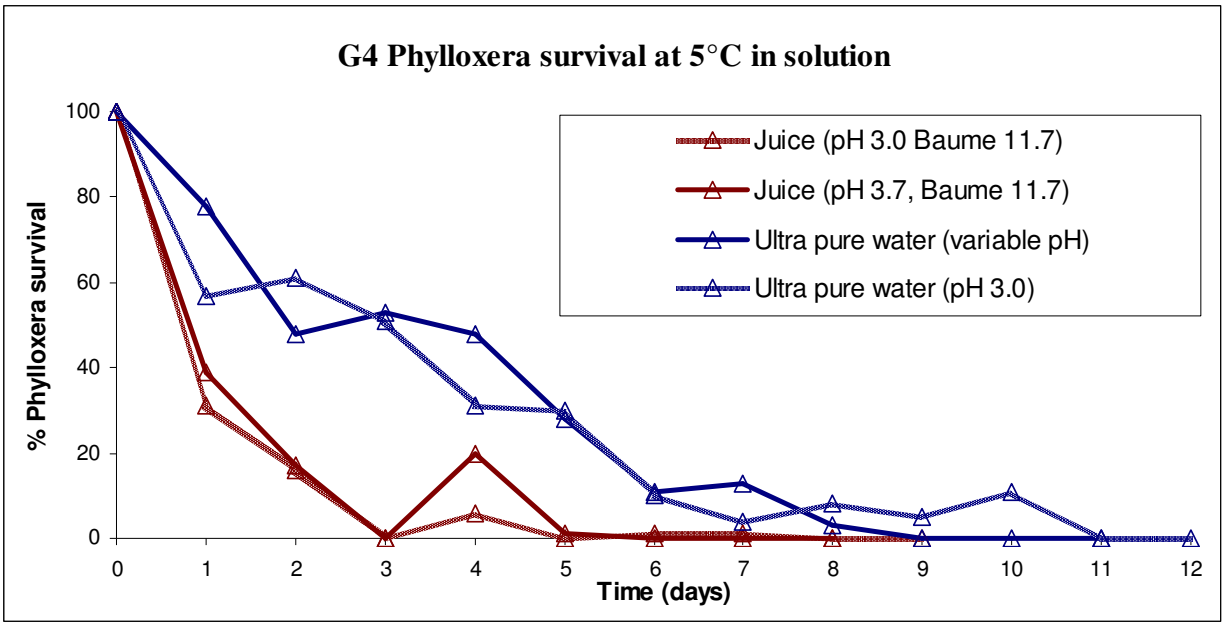


Figure 49 Influence of white juice pH on first instar G4 phylloxera crawler survival.
Temperature adjusted to 5ºC; Baumé adjusted to 11.7. n=100.

White juice and Baumé

Phylloxera survival at 5ºC in Chardonnay juice, adjusted to a Baumé of either 11.1 or 14.1 with pH standardised at 3.4 was compared with survival in a control solution of glucose/fructose at a Baumé of either 11.0 or 14.0 (Fig. 50).

Baumé reduced survival on control treatments to a maximum of 7 days compared to 10 days in the water control. This is a similar duration to previously reported data on phylloxera survival in the absence of diet under ambient conditions (Kingston *et al*., 2007). Although the combination of juice and Baumé did reduce survival initially (i.e. in the first 3 days of the trial) compared with the Baumé adjusted glucose/fructose control solutions, some phylloxera still survived for up to 7 days (Fig. 50) in both Baumé treatments. This indicates that some other characteristic of white juice, other than Baumé alone, reduces phylloxera survival. Changing the Baumé level from 11 to 14 had no significant impact on survival in both the glucose/fructose and juice treatments.

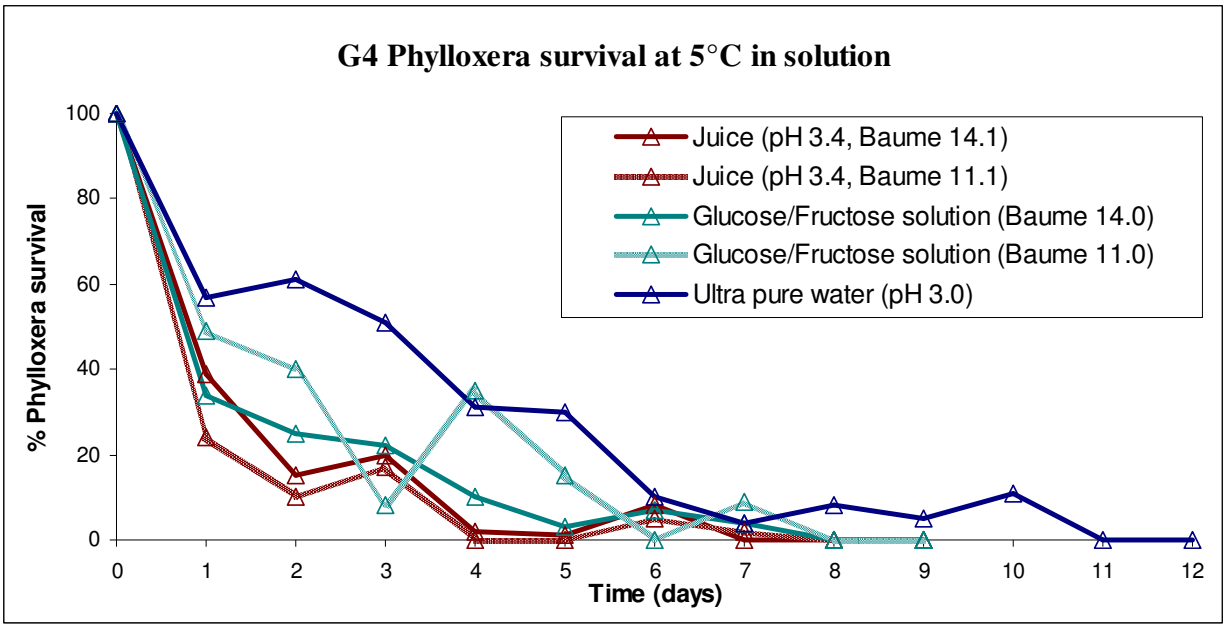


Figure 50 Influence of white juice Baumé level on first instar G4 phylloxera crawler survival. *Temperature adjusted to 5ºC; pH adjusted to 3.4. n=100*

White juice and cold temperature

Survival of phylloxera in white juice, glucose/fructose solution or water was compared at 2ºC, 5ºC and 10ºC (Figs. 51-53). At 10ºC phylloxera survived for up to 10 days in water with pH adjusted to 3.4 or variable pH. In white juice, at 10ºC, 100% mortality was achieved at 8 days. At 5ºC phylloxera similar trends in survival were observed. Phylloxera survived for up to 10 days in water. Although the proportion of phylloxera surviving in the controls was lower than at 10 ºC. In the white juice treatment 100% mortality was achieved again at 8 days.

The lowest temperature tested was 2ºC which had the most significant impact on phylloxera survival. 100% phylloxera mortality was achieved by day 2 in white juice. At day 7 in the water control there was still some phylloxera survival (even though the proportion surviving in the water control was lower than at 5ºC). This indicates that cold temperature (i.e. 2ºC) when combined with juice has a significant impact on phylloxera crawler survival in white juice.

White juice and sulphur

Survival of phylloxera in either white juice, or water with sulphur dioxide (in the form of added potassium metabisulphite (PMS)) was compared at 10ºC and 2ºC only (Figs. 54 and 55). At 10ºC, some phylloxera mortality still survived after 8 days in the water + sulphur control. In contrast when sulphur was not added to the water control over 30% of the insects survived up to 9 days. This suggests that sulphur in water significantly reduces the proportion of phylloxera surviving.

In white juice with added sulphur insects only survived for 6 days compared to 7 days in white juice with no added sulphur indicating that sulphur may have a similar efficacy in water as in white juice but not an additive effect.

At 2ºC all phylloxera in the water control, with added sulphur, 100% phylloxera mortality was achieved within 4 days whereas in the absence of sulphur phylloxera survival in water was at least 7 days. In white juice (+ PMS) 100% mortality was achieved at 2 days, which was effectively the same duration as white juice with no added PMS. This indicates that there may be some interaction with sulphur with white juice which affects its efficacy towards phylloxera.

Overall the combined treatments of white juice, added sulphur and 2ºC had the most marked effect on phylloxera survival.

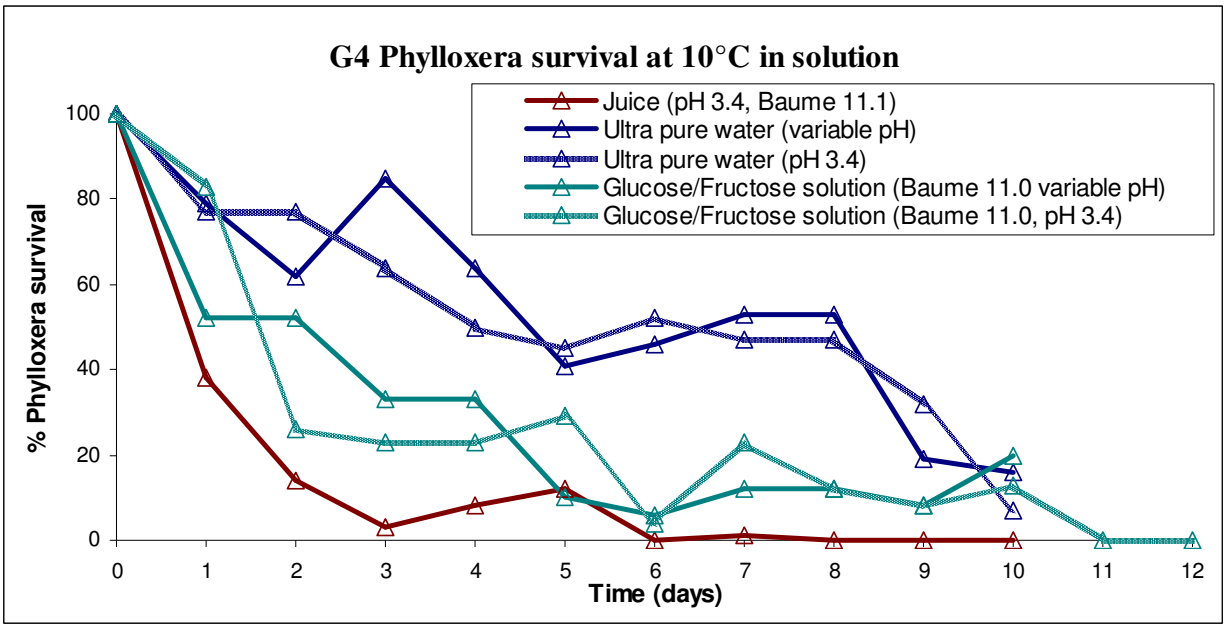


Figure 51 Influence of solution on first instar G4 phylloxera crawler survival.

Temperature adjusted to 10ºC; pH adjusted to 3.4 and Baumé to 11.5º, n=100

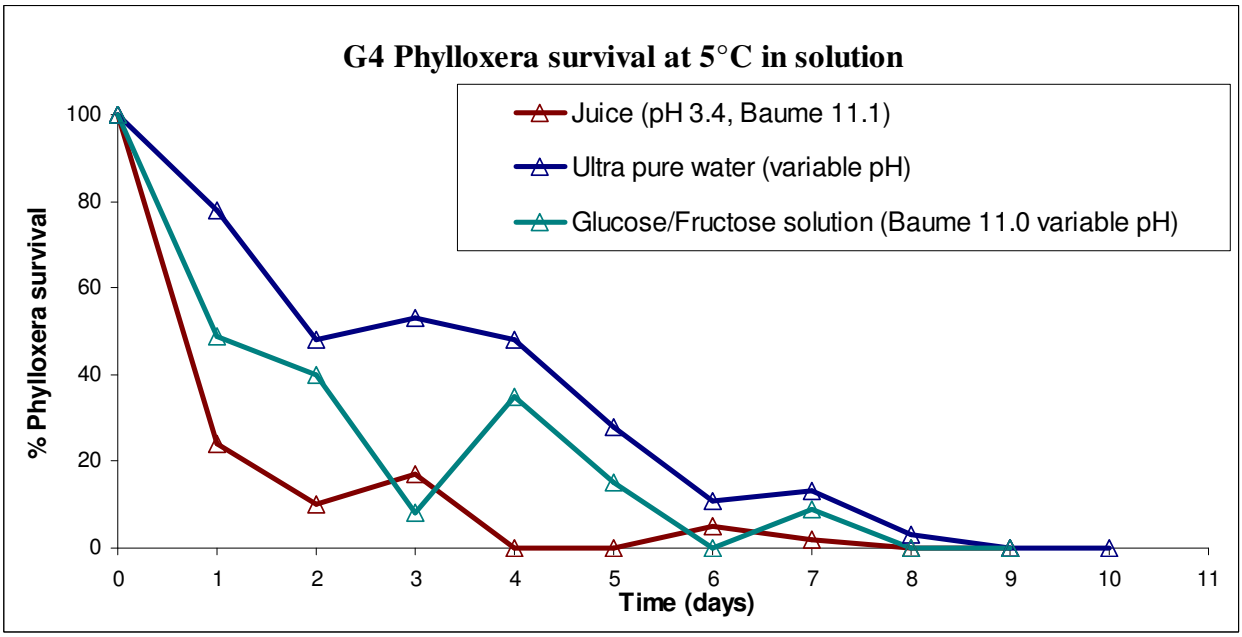


Figure 52 Influence of solution on first instar G4 phylloxera crawler survival.

Temperature adjusted to 5ºC; pH adjusted to 3.4 and Baumé to 11.1º, n=100.

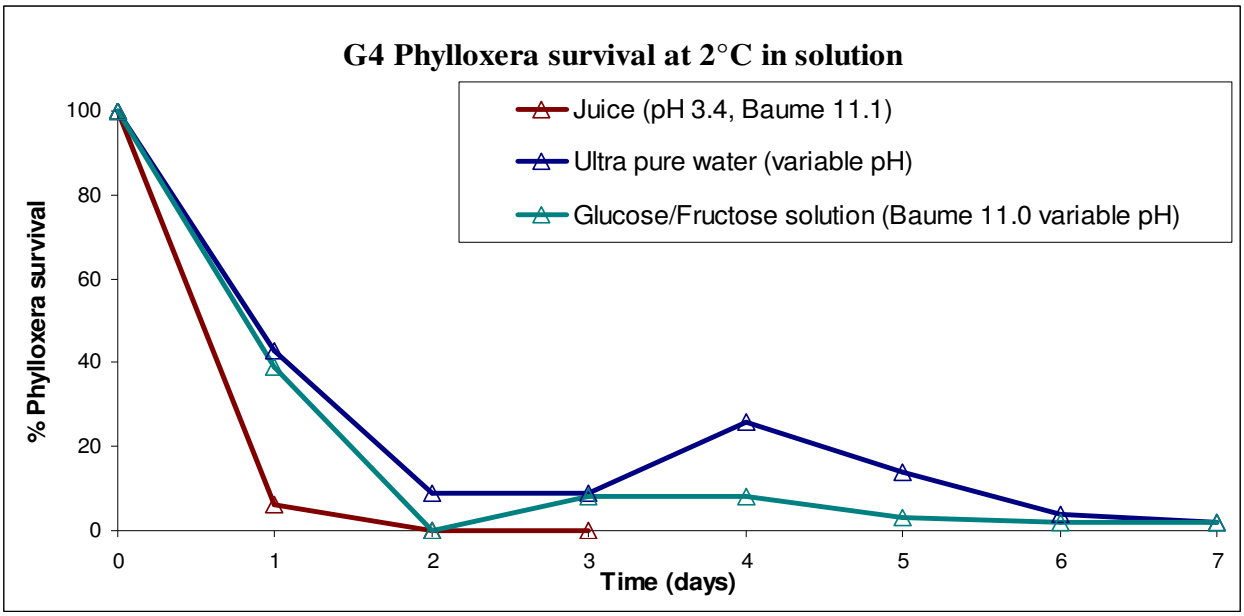


Figure 53 Influence of solution on first instar G4 phylloxera crawler survival.

Temperature adjusted to 2ºC; pH adjusted to 3.4 and Baumé to 11.5º, n=100.

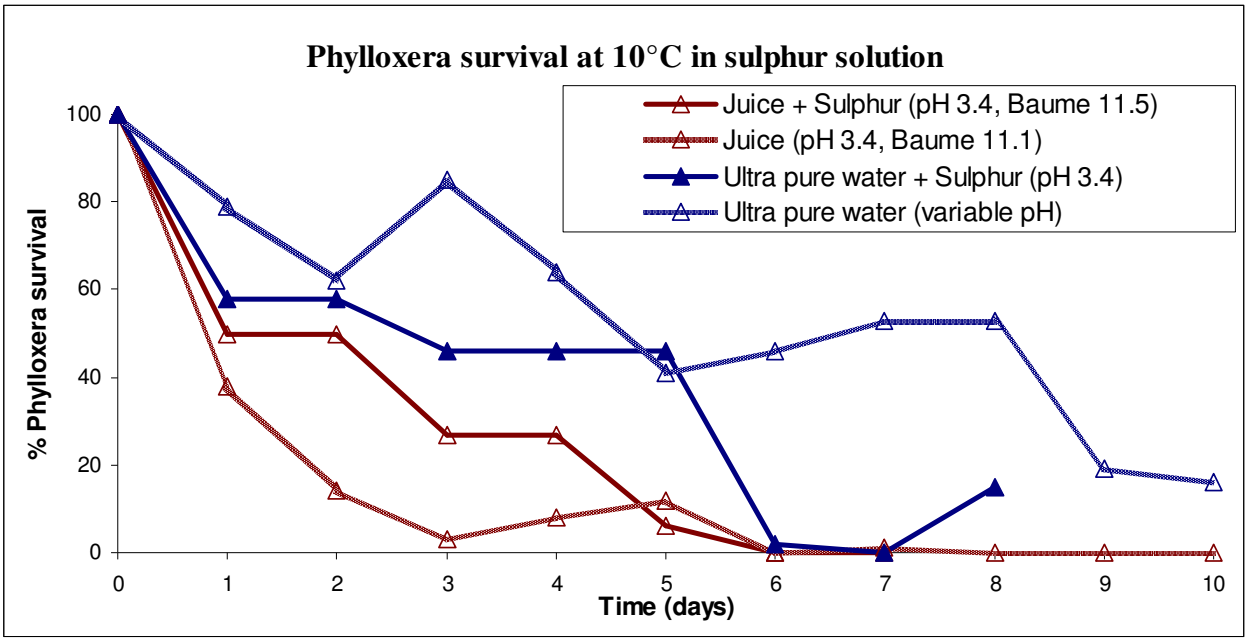


Figure 54 Influence of sulphur in solution on first instar G4 phylloxera crawler survival. *Temperature adjusted to 10ºC; pH adjusted to 3.4 and 11.5º Baumé, n=100.*

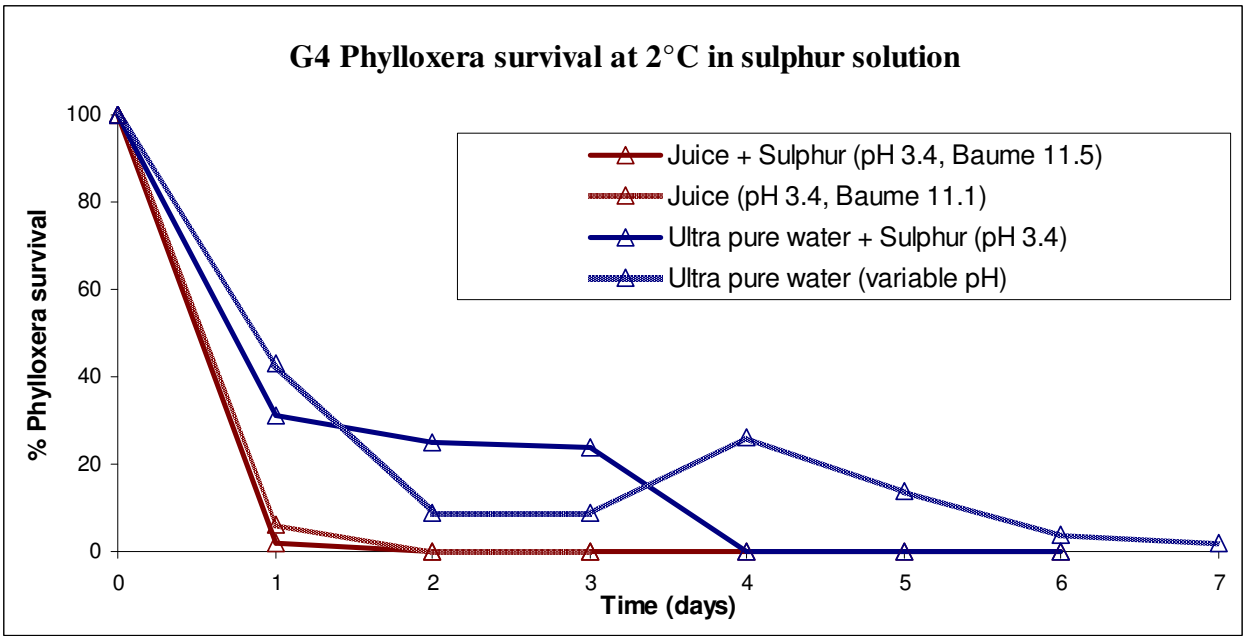


Figure 55 Influence of sulphur on first instar G4 phylloxera crawler survival. *Temperature adjusted to 2ºC; pH adjusted to 3.4 and Baumé to 11.1º, n=100.*

Discussion

In this study we validated the upper time limits for first instar survival of a single highly virulent phylloxera genetic strain, G4, in several treatment conditions. These conditions could potentially impact on disinfestation treatment and quarantine protocols for white juice. It was observed (as has been previously noted) that phylloxera is quite resilient to drowning and can survive for several days in a range of solution types and conditions. Phylloxera can survive in water alone for up to 10 days. Overall acidity and Baumé, within the ranges tested, had minimal impact on phylloxera survival. However white juice, sulphur dioxide and cold temperature did increase mortality.

A 5ºC in varying pH (3.0 to 3.5) and Baumé (11.0 to 14.1) conditions the survival of phylloxera crawlers was only marginally reduced in control treatments. However, when these conditions were combined with white juice phylloxera survival was reduced further. This indicates that some, as yet unidentified, characteristic of white juice other than Baume and pH may have a detrimental effect on first instar phylloxera survival.

Phylloxera active stages have previously been shown to survive in the absence of a food and when submerged in water can survive for over a week at 5ºC and 25ºC (Korosi *et al*., 2009). This is may be partially due to the ability of phylloxera to store food due to their specialised digestive system (Andrews *et al*., 2012) and also the insects' ability to overwinter as first instar crawlers, probably by reducing their metabolic rate, when temperatures are reduced or suboptimal. In the experiments reported here temperature had a significant effect on phylloxera survivalPhylloxera survival in water and juice at 5ºC and 10ºC was up to 10 days, indicating that cold treatment of white juice between 5-10ºC would be a relatively ineffective method of disinfestation for phylloxera. However, at 2ºC in juice 100% mortality was achieved at 2 days indicating that this lower temperature could be an effective disinfestation treatment.

Addition of sulphur dioxide in the form of potassium metabisulphite, in solution, had an impact on mortality at both 10ºC and 2ºC, when combined with white juice, but interestingly the presence of sulphur in water alone had a more marked impact on phylloxera survival. This suggests that the efficacy of sulphur towards phylloxera is affected by the presence of white juice. Sulphur dioxide is used as a disinfestation protocol for table grapes (but not in solution) and has been shown to effective at 5.2ppm at 5ºC for 36h (Buchanan 1990). It is currently recommended as a disinfestation procedure for table grapes at 970g/kg sodium metabisulphite in sulphur pads (Procedure F - National Phylloxera Management Protocols; NVHSC 2009) but prior to this report had not previously been tested in white juice.

Recommendations

1. Based on our study the existing protocols for juice disinfestation could potentially be modified for white juice based on cold temperature treatments. However, this would require endorsement by an accredited national viticulture biosecurity body. In the absence of the National Vine Health Steering Committee (disbanded in 2010) it is unclear at this stage which body would endorse any changes to the National Phylloxera Management Protocol.

2. There have been 83 different genetic strains identified in Australia (Umina *et al*., 2007) and in our study we only tested one highly virulent clonal lineage. Upper thermal limits of phylloxera survival have recently been shown to differ between at least two phylloxera clonal lineages (Korosi *et al*., 2011a). Ultimately further studies should be conducted to determine impacts of juice conditions on more than one phylloxera clonal line to further elucidate the potential efficacy of cold heat treatment protocols for white juice disinfestation.

3. We have developed some simple protocols for assessing the impact of disinfestation treatments on phylloxera genotypes, which could be modified for other potential treatments. A review of the National Phylloxera Management Protocol may highlight any other areas of the protocol which may potentially need refinement.

4. It is clear from these studies that white juice alone has a significant effect on phylloxera survival yet it is not clear whether this is due to physical or chemical properties of the white juice. If it is due to the presence of chemical compound (such as flavanoids or tannins) in the juice then identification and isolation of these compound(s) would be useful to determine their efficacy as antimetabolites.

CHAPTER 6

TARGETTED PHYLLOXERA DETECTION

Output: *Comparative analysis of phylloxera early detection systems*

Summary

In Australia, early detection of grapevine phylloxera is critical for the sustainability of the Australian viticulture industry which is predominantly planted on phylloxera-susceptible ungrafted *V. vinifera* The current range of conventional detection methods used include (i) ground surveys involving systematic sampling and visual examination of grapevine roots, either as a stand-alone system or combined with the use of (ii) multispectral aerial imagery, which relies on detection of 'weak' spot's in the vineyard resulting from the expression of non-specific, plant stress in the vegetation. These two methods, although widely used, are costly, labour intensive and have some potential for human error.

Other methods being evaluated include the use of chemical fingerprinting (Benheim *et al*., 2011), a soil-based DNA probe (Herbert *et al.,* 2008) and phylloxera emergence traps (Powell *et al*., 2009). However, there is also a need to develop biophysical descriptors that directly indicate the relative potential susceptibility of viticultural areas to phylloxera outbreaks. This is so that targeted and intensified surveillance may be conducted in areas where phylloxera is most likely to establish.

Grapevine phylloxera is recognised as a pest of significance to the viticulture industry world-wide, yet little research has been conducted to develop a system that optimises its early detection and subsequent control. There have been several phylloxera detections in Australia since the mid-1990s (Chapter 7; Figure 72), including in the Yarra Valley wine region in

2006-08 and Mansfield 2010 (Chapter 1), which, due to the level of infestation and vine damage detected, where thought to have been present in the vineyards for several seasons prior to detection. This highlights the need to improve sampling strategies and thereby optimise detection methods.

As part of an evaluation strategy for targeted phylloxera detection monitoring was conducted to compare the use of conventional ground surveys, emergence traps and a phylloxera-specific soil DNA probe in an infested vineyard in the Yarra Valley over three successive growing seasons. A comparison of the efficacy of these sampling techniques was made in two known infested blocks in the vineyard. In addition, a three season pilot study in targeted phylloxera detection was conducted in a high risk vineyard in the Yarra Valley.

To develop a targeted sampling approaching, in addition to direct phylloxera sampling, two indirect remote sensing techniques were utilised. Because phylloxera has an uneven spatial distribution in a vineyard two remote sensing techniques were employed, (i) measuring soil electrical conductivity and (ii) measuring NDVI index, to improve the sampling protocol. The two techniques soil electromagnetic induction (EM38) surveys and 'Greenseeker' (NDVI) vegetation surveys were conducted to provide background data to optimise and compare the sensitivity of three primary detection methods root observation, emergence traps and soil DNA probe. NDVI and EM38 data, as previously reported (Bruce et al., 2009), may indicate relationships between soil properties, vine vigour and phylloxera abundance.

In the first two seasons, representing ‘early’ detection, both the DNA probe and emergence traps provided an accurate quantitative assessment of phylloxera abundance in soil adjacent to infested grapevines. Their efficiency compared to using conventional ground surveys was influenced by sampling date.

Before either the DNA probe and/or the emergence trapping technique could be used as an alternative to conventional ground surveying they will require further validation under a range of soil conditions, in a range of grafted and ungrafted vineyards and in vineyards with relatively low abundance of less virulent phylloxera genetic strains. As well as relative

efficacy, an economic assessment will also be required to determine which detection technique or techniques are most cost effective.

Introduction

Conventional ground surveying for phylloxera detection relies on examination of the root system for phylloxera presence combined with examining the grapevine canopy for symptoms of vine stress (Fig. 2; Chapter 1). Symptoms of phylloxera infestation are easily confused with other vine stress reactions under field conditions, such as extreme temperatures or sustained water deficit (Blanchfield *et al*., 2006).

Current early detection systems for phylloxera, including multispectral imagery and infrared photography (Renzullo *et al*., 2007), still require systematic ground surveying and are both labour intensive and weather dependent. Relying solely on remotely sensed images for phylloxera detection is also problematic in areas of high vigour, if the infesting phylloxera genotype happens to be of low virulence or in grafted vineyards as classical stress signals may not be clearly expressed by the vines. While emerging technologies such as spectral fingerprinting (Renzullo *et al*., 2006), chemical fingerprinting (Blanchfield *et al*., 2006; Tucker *et al*., 2007; Benheim *et al*., 2011), trapping (Powell *et al*., 2009) and a soil DNA-probe (Herbert *et al*., 2008) are promising methods of indirect and direct phylloxera detection, validation of these techniques under field conditions has been limited. There is also a need for the development of a targeted phylloxera detection approach to identify phylloxera 'hotspots' to facilitate the implementation of these new technologies more effectively in commercial situations.

This Chapter focuses on a comparative study of conventional and novel detection methods under commercial vineyard conditions and how they might be utilised in an integrated approach for phylloxera detection in the future. In an integrated phylloxera detection system there are two synergistic approaches for detection; (i) direct or primary detection (where the phylloxera or its DNA are directly assessed both qualitatively and quantitatively) and (ii) indirect or secondary detection where symptoms of phylloxera induced

stress or suitable habitats for phylloxera establishment are targeted (Powell, 2008; 2012). Primary detection methods include ground surveying (root examination), a molecular probe and insect traps whereas secondary detection methods utilise remote sensing.

Conventional ground surveys have been used in Victoria, NSW and Queensland for decades and involve examination of the root system of grafted and ungrafted *V. vinifera* for the presence of phylloxera-induced root damage and the insect colonies. These surveys are generally conducted in the spring and summer when phylloxera populations are at their peak on the root system of infested grapevines (Powell *et al*., 2000) and hence more readily detected.

A phylloxera-specific DNA probe was developed originally as part of a CRCV-funded PhD research project (Herbert *et al*, 2008). The probe that was developed was first validated in a limited study under laboratory and field conditions at a single vineyard site, over a single season, in the Yarra Valley, Victoria in 2007 (Herbert *et al*., 2008).

A conventional, grower-friendly, insect trapping method, called the emergence trap, has been modified for collection of dispersive stages of phylloxera, namely first instar or 'crawlers' and alate or winged adults. This method has been used successfully in a number of studies to monitor the population dynamics of grapevine phylloxera in Victoria on both grafted and ungrafted grapevines (Powell *et al*., 2003, 2009; this report Chapter 1) and tested once as a potential detection option (Herbert *et al*., 2008).

Secondary detection methods include soil and vegetation surveys. Apparent soil electrical conductivity (ECa) is measured using a two dimensional EM38 soil survey method. It has previously been trialled over a single season in Victoria in combination with emergence trapping (Bruce *et al*., 2009).

NDVI data was collected using a side-orientated, 'Greenseeker', reflectance sensor (NTech Inc., USA), mounted on an all-terrain vehicle (ATV), with an in-built light source reflectance of plant canopy into two wavelengths. As the instrument passes the grapevine canopy a data logger records red and near infra-red. This system has also been previously trialled over a single season in Victoria in combination with emergence trapping where it

appeared more sensitive than multispectral data collected using airborne sensors (Bruce *et al*., 2009) as has been widely advocated and used in South Australian phylloxera surveys.

Methods

Site selection

Site 1 – Comparison of Detection Methods in a Known Phylloxera Infested Vineyard

Following a detection of G1 phylloxera in a Yarra Valley vineyard in 2008 two infested vineyard blocks, A and D, were selected for comparison of three primary detection techniques and evaluation of two secondary detection techniques, for the first time over three successive seasons. Comparative studies on the efficacy of the DNA probe, emergence traps and ground surveys are described. The secondary techniques used were soil electrical conductivity (using EM38) and canopy vigour (using Greenseeker™) sensing to aid in identification of 'hot spots' to potentially reduce the number of required primary detection samples by indicating areas of high phylloxera 'establishment potential'.

Site 2 –Targeted Detection Pilot Study in a Phylloxera Risk Vineyard

Following a further detection of G1 phylloxera in the Yarra Valley in December 2010 an EM38 survey was also conducted at a second infested field site (Site 2). The commercial vineyard had removed the known phylloxera infested block of *V. vinifera* cv. Chardonnay prior to the survey being conducted. Once removed the remaining vineyard area was selected as a potential phylloxera-risk site for further monitoring using a targeted surveillance approach. At this site an EM38 survey was conducted of the whole 30ha property in early December 2011 and strategic placement of emergence traps occurred in late January 2011 in selected blocks, based on EC ranges of interest, as identified from the comparative detection study conducted at Site 1.

Site 1

Remote sensing - baseline imagery collection

In the first season of the study (2008-2009), at Site 1, baseline ECa maps and NDVI maps were collected in February 2009 (as described in Bruce *et al*., 2009, 2011) and limited testing of primary detection methods were conducted in both infested ungrafted *V. vinifera* 'Chardonnay' blocks designated Block A and Block D, following a detection of phylloxera on the property in December 2008.

Emergence traps (seasonal monitoring)

Season 1 (2008/2009)

Confirmation of G1 phylloxera infestation by root examination and DNA typing in a single vineyard block (A) at Site 1, Yarra Valley, Victoria occurred in late 2008. Emergence traps were initially placed in the infested block to monitor phylloxera population abundance and determine spread around the initial visual weak patch of grapevines.

A second block (D) at Site 1 was also confirmed to be infested with G1 after emergence traps were placed in a suspect weak patch from visual observations. In block A, 51 vines and in Block D, 40 vines were initially monitored using emergence traps (Fig. 3; Chapter 1). Phylloxera was monitored on a monthly basis in both blocks from December 2008 to May 2009 representing 6 sample collection dates per block. All trap locations were geo-referenced using a Trimble GeoXT GPS (Trimble Navigation, Queensland) and post-processed using Land Victoria GPSnet data for sub-metre accuracy.

Season 2 (2009/2010) and Season 3 (2010/2011)

Seasonal monthly monitoring of emergence traps was continued in seasons 2 and 3 on selected infested grapevines in each infested block. All trap locations were geo-referenced using a Trimble GeoXT GPS (Trimble Navigation, Queensland) and post-processed using Land Victoria GPSnet data for sub-metre accuracy.

DNA Probe - summer sampling

At site 1 soil cores were collected from infested and suspected infested vines across both blocks A and D on 24th February 2009. Four soil cores were taken per 'trap referenced vine' from within a 30cm radius of the vine trunk spread evenly to give a good representation, and to total no less than 500g processed weight. Each core was 20cm (depth) x 2.5cm (diameter) in size. A total of 328 cores were collected representing 81 vines.

Soil samples were processed at the DPI Victoria-Rutherglen Centre under quarantine conditions, and oven dried at 50°C for 48hrs to devitalise the phylloxera for transport interstate to South Australia, as required by Plant Health Certificate (issued by Plant Standards Victoria) and Import Permit (issued by Department of Primary Industries and Resources of South Australia (PIRSA)). This temperature treatment and duration has not been found previously to affect the sensitivity of the DNA testing.

Soil was weighed into 500g samples and dispatched under quarantine regulations to the South Australian Research and Development Institute (SARDI), Adelaide where they were tested for the presence of phylloxera DNA (detection sensitivity is 1 phylloxera per 200g soil). In total 82 samples were screened including one no-phylloxera control sample taken from a non-vineyard location.

DNA Probe - winter sampling

To assess the potential for detection of phylloxera during its winter dormancy period, when it is relatively inactive and cannot be detected using emergence traps, the DNA probe was selectively tested at site 1. Soil cores were collected in season 2 on 25th August 2010 from 18 randomly selected infested vines within Blocks A and D. Soil core sample locations were selected on the basis of emergence trap and ground survey data collected in February of both seasons. Core samples were collected and analysed as described for Season 1.

Detection technique - Comparative study

In seasons 2 and 3 at site 1 a systematic ground survey was conducted using a grid pattern across blocks A and D (Fig. 56) in conjunction with the use of additional emergence trap and DNA probe sampling. In accordance with the ground survey procedure (NVHSC, 2009) developed by DPI Victoria in each of the two experimental blocks, the roots of grapevines were examined by a ground survey team using a x10 hand lens. The systematic sampling procedure involved excavation of the root system of a single grapevine in every third row and every fifth panel. In both seasons emergence traps were also placed at each of the vines (in early January) examined by the ground survey teams, and samples examined after one month for phylloxera presence.

Soil cores were collected in seasons 2 and 3, on 30th March 2010 and 2011 respectively from infested vines within Blocks A and D. Core location was selected on the basis of emergence trap and ground survey data collected in February of both seasons. Cores were taken as for Season 1 and the total number of cores collected was expanded in seasons 2 and 3 as phylloxera had spread further within the blocks.

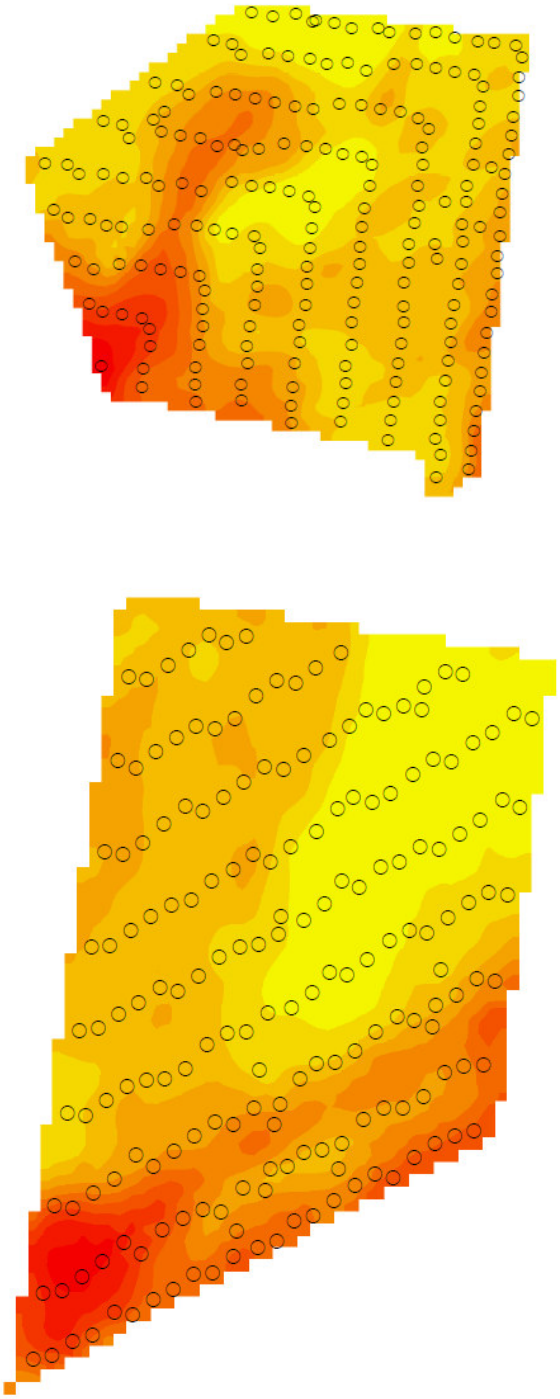


Figure 56 Grid sampling points overlapping EM38 baseline map at site 1 in Blocks A and D were used to compare detection methods.

In every third row and every fifth panel a single vine was sampled using three primary detection techniques: emergence traps, DNA soil probe and ground surveying. This grid-based survey protocol was used in season 2 and season 3. Open circles represent sample points.

Results

Season 1 (2008/2009)

Phylloxera distribution and soil electrical conductivity

Figures 57-58 and 61-62 show maps of soil apparent electrical conductivity (ECa), derived from EM38 surveys, of Block A and D and the sample points for phylloxera detection using, either emergence traps or the soil DNA probe.

In block A, all phylloxera-positive samples, whether emergence trap or DNA probe, were detected within the 16.1-19.5 mS/m range (Figs. 57, 58 and 59). In block D all phylloxera positive samples were found within the 14.3-24.6 mS/m range. A maximum 479 phylloxera, were collected in a single emergence trap over the sampling season (data not presented). In both blocks there was a significant correlation between phylloxera abundance in traps and levels detected using the DNA probe and therefore quantitative agreement between these two sampling methods ($p<0.001$; Fig. 60).

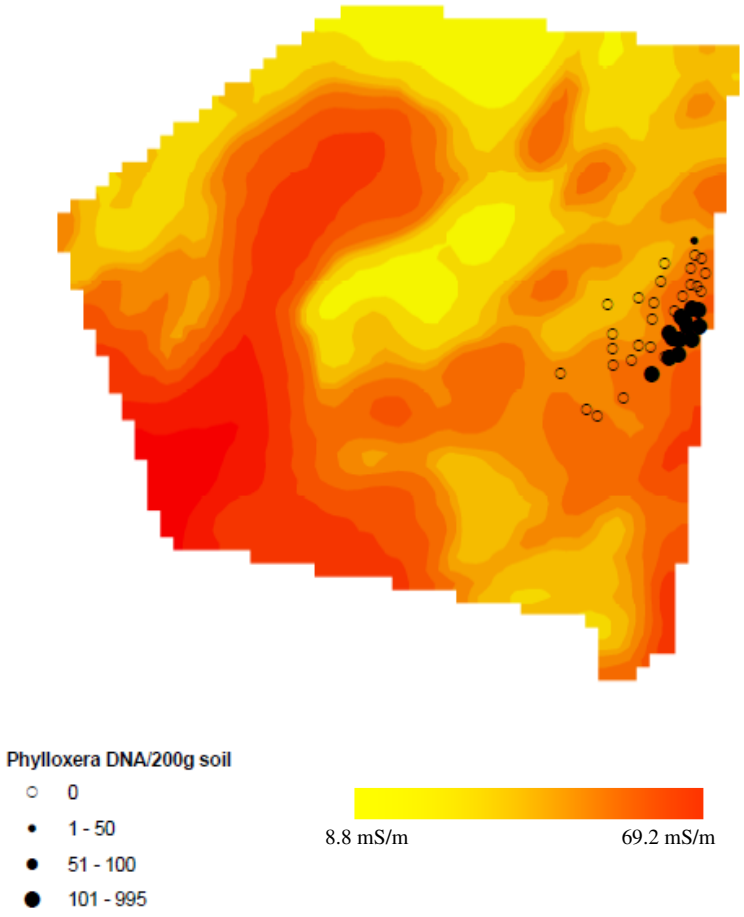


Figure 57 Block A electrical conductivity map derived from EM38 survey showing detected levels of phylloxera per 200g soil (Season 1) using the DNA probe in February 2009.

All phylloxera-positive samples were found within the 16.1-19.5 mS/m range.

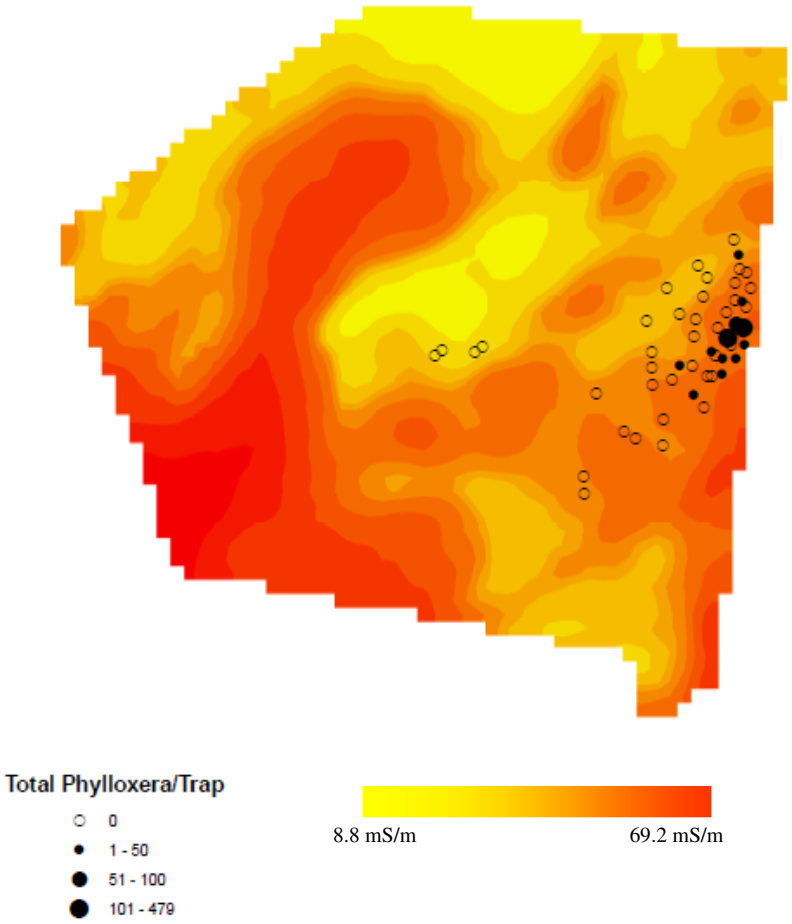


Figure 58 Block A electrical conductivity map derived from EM38 soil survey showing detected levels of phylloxera per emergence trap over the sampling period (January-February 2009) (Season 1).

All phylloxera-positive samples were detected within the 16.1-19.5 mS/m range.

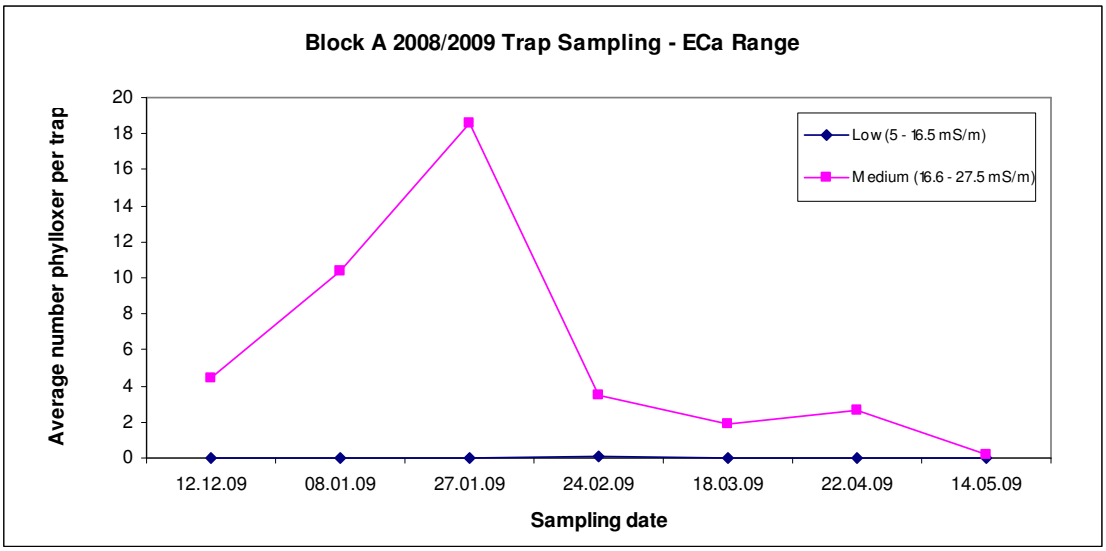


Figure 59 **Block A (site 1) showing mean seasonal abundance of phylloxera in emergence traps over the sampling period (December 2008-May 2009) (Season 1).** *A medium range is shown and within this range all phylloxera-positive samples were detected within the 16.1-19.5 mS/m range.*

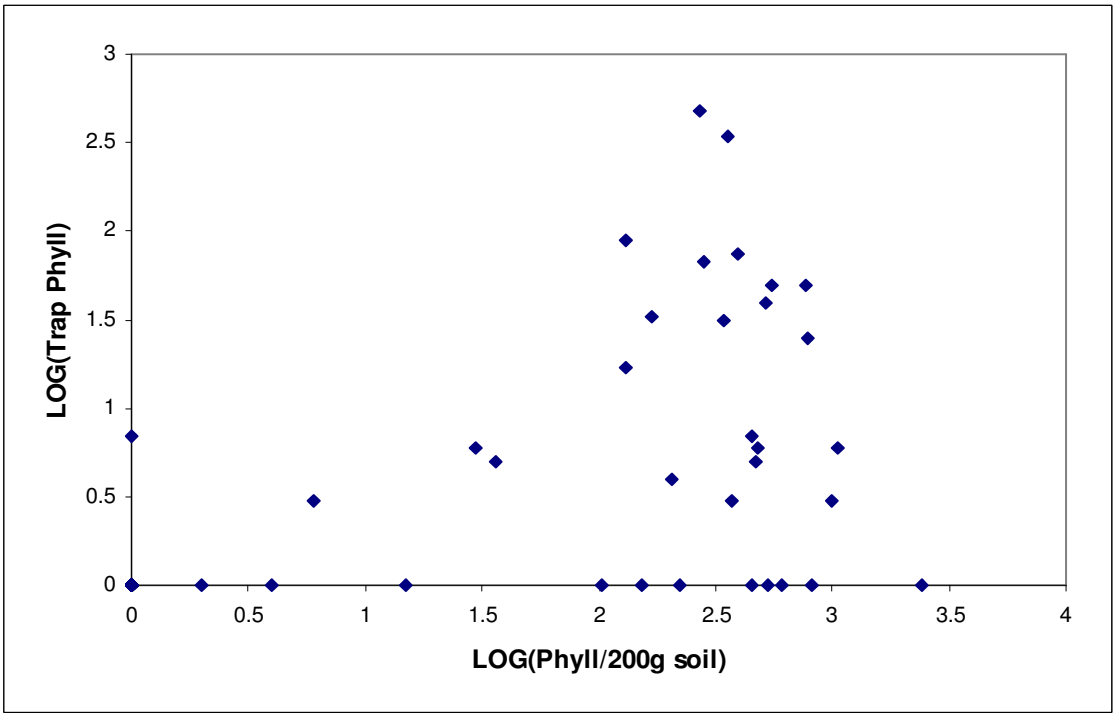


Figure 60 **Number of phylloxera detected in emergence traps plotted against the amount of phylloxera DNA detected at site 1 using the soil DNA probe in February 2009.**

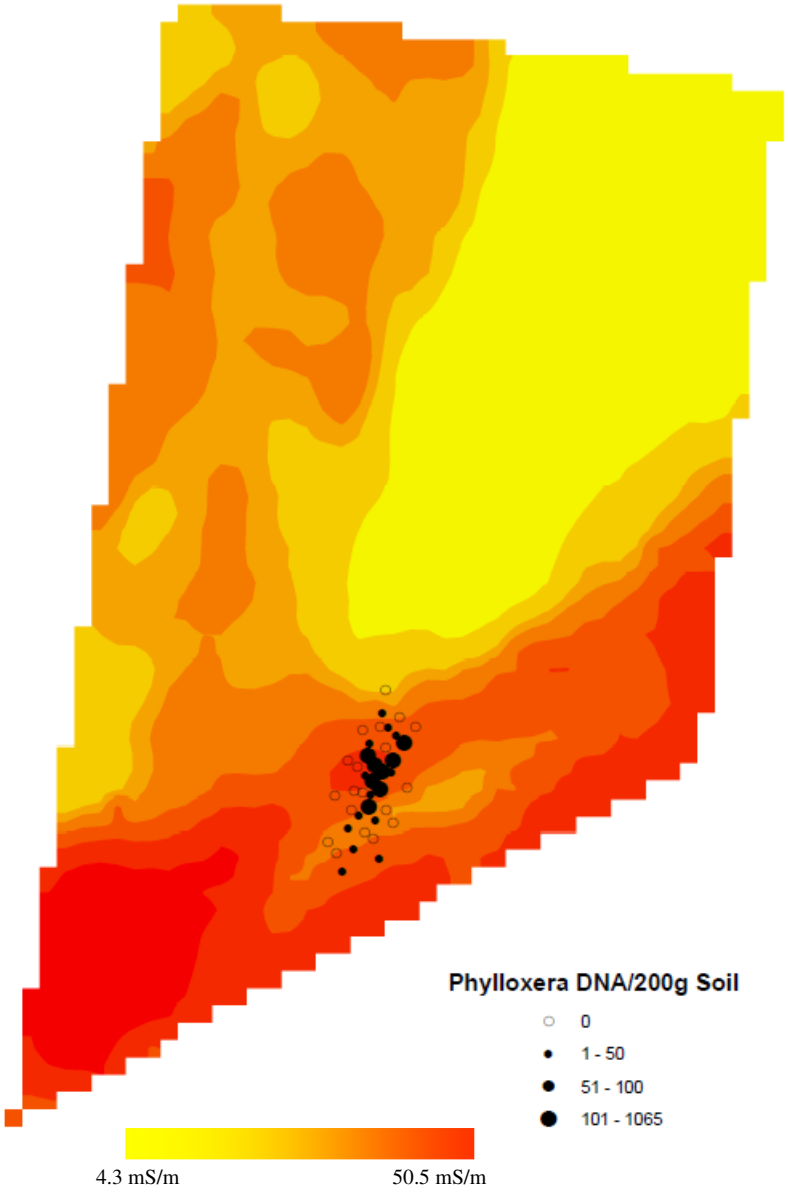


Figure 61 Block D electrical conductivity map derived from EM38 survey showing detected total levels of phylloxera per 200g soil (Season 1) using the DNA probe in February 2009.

All phylloxera-positive samples were found within the 14.3-24.6 mS/m range.

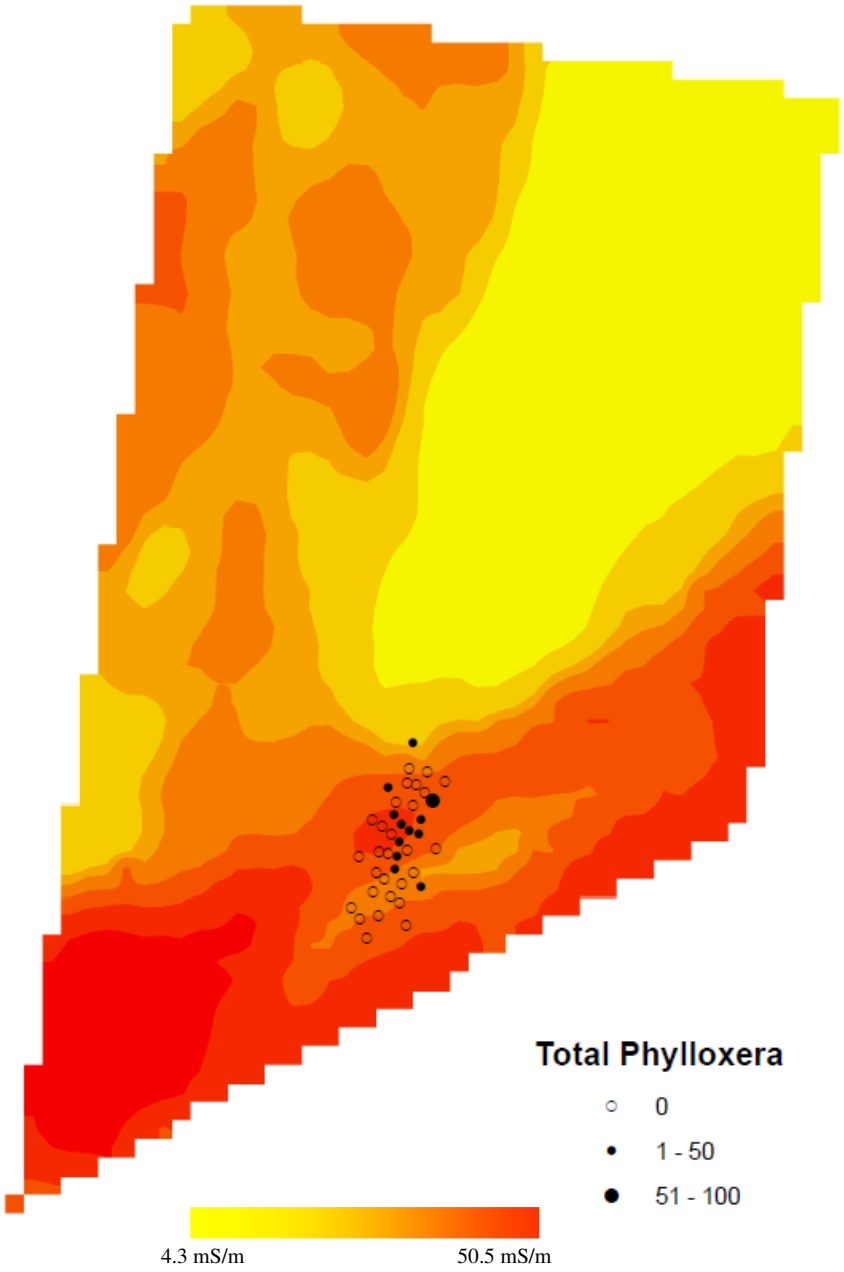


Figure 62 Block D electrical conductivity map derived from EM38 survey showing detected total levels of phylloxera per emergence trap over the sampling period (January-February 2009) (Season 1).

All phylloxera-positive samples were found within the 14.3-24.6 mS/m range.

Phylloxera detection and vegetative indices

A 'Greenseeker' device was used to map the NDVI vegetative index of the grapevine canopy. In season 1 all phylloxera-positive samples, whether emergence trap or DNA probe, were detected within the 0.71-0.85 NDVI range (Figs. 63 and 64).

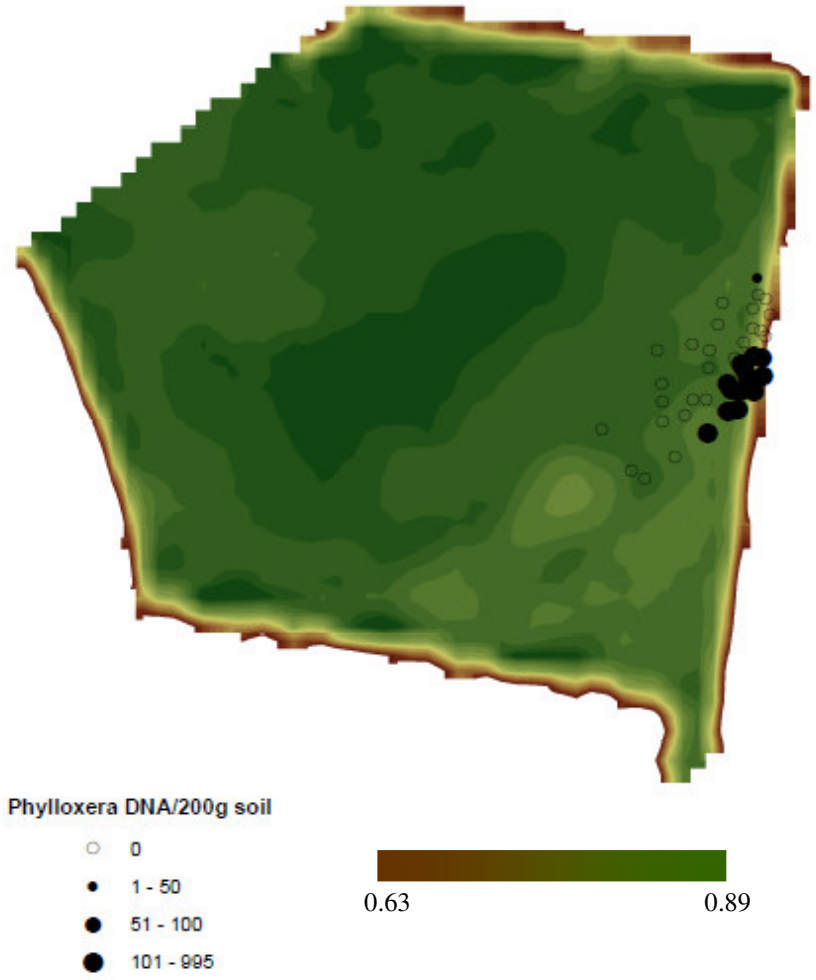


Figure 63 Block A (site 1), NDVI map derived from 'Greenseeker' survey showing detected levels of phylloxera per 200g soil (Season 1) using the DNA probe in February 2009.

All phylloxera-positive samples were found within the 0.71-0.85 range.

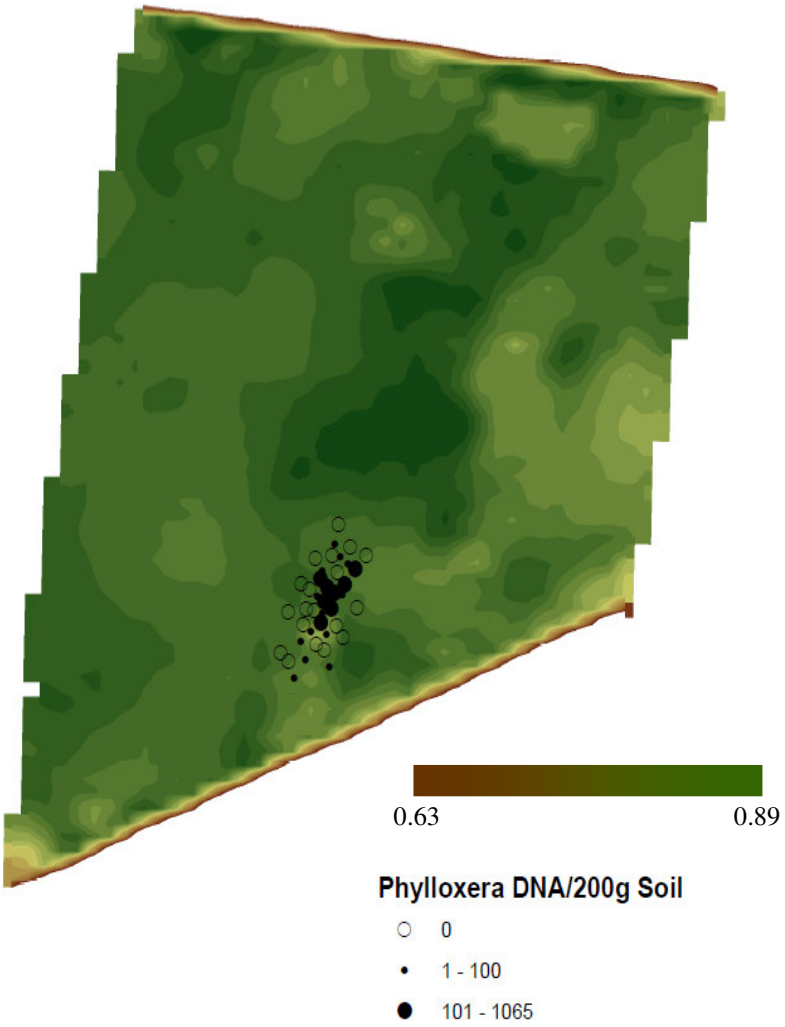


Figure 64 Block D (site 1), NDVI map derived from 'Greenseeker' survey showing detected levels of phylloxera per 200g soil (Season 1) using the DNA probe in February 2009.

All phylloxera-positive samples were found within the 0.81 – 0.84 range.

Emergence traps (seasonal monitoring)

Monitoring of phylloxera emergence from the soil in each infested block was conducted using emergence traps over three seasons. The monitoring was conducted to determine peak population abundance (which can differ between seasons depending on soil temperature).

Population abundance in season 1 was relatively low in blocks A (Fig. 65) and D (data not presented) with a maximum total of 479 phylloxera in Block A traps and 100 in Block D traps. In season 2, populations were more abundant with a maximum total per trap of 1111 and 966 recorded in Blocks A and D respectively. Peak phylloxera abundance occurred in January-February. In season 3 traps numbers were lower than season 2 which may be due to either excessive root damage caused by higher phylloxera abundance in the preceding season or some other environmental affects, such as temperature or rainfall, affecting emergence.

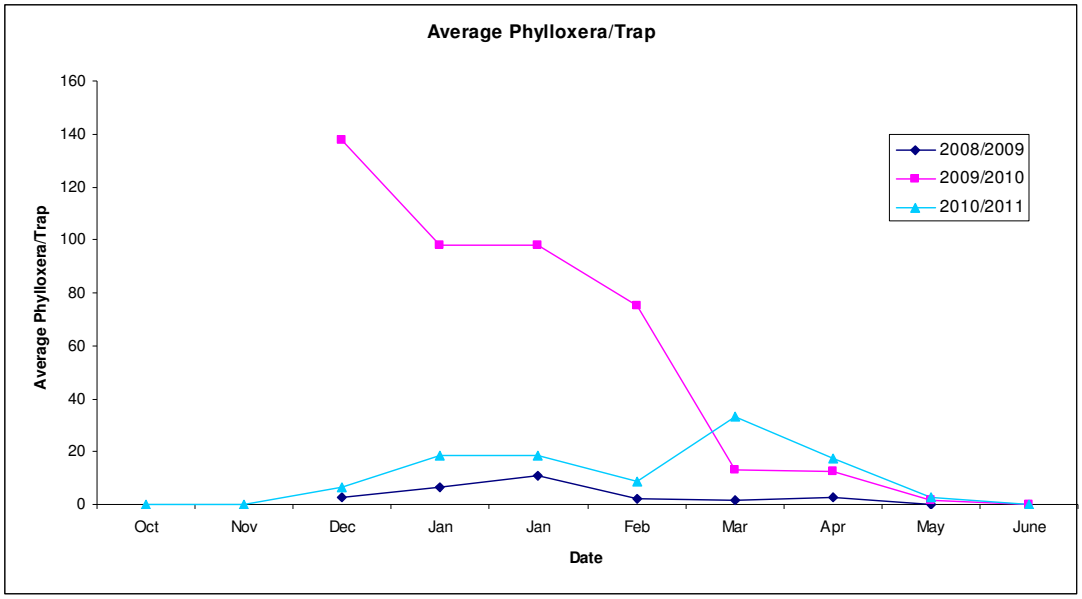


Figure 65 Seasonal abundance of above-ground first instar phylloxera (mean per emergence trap per vine) on ungrafted *Vitis vinifera* 'Chardonnay' in Block A (site 1) during the 2008/9, 2009/10 and 2010/11 growing season at the Yarra Valley site 1.

Comparative Detection Study - Season 2 (2009/2010)

Figures 66-67 show maps of soil electrical conductivity derived from EM38 surveys of Blocks A and D, ground survey data and detected levels of phylloxera per 200g soil and in emergence traps. Open circles indicate vines where phylloxera was not detected.

In block A, detections by the ground survey team were similar to those detected by DNA testing, yet both showed less than detections by emergence trapping (Fig. 66). The relatively lower number of detections by DNA probe sampling, compared to emergence trapping could be due to the DNA samples being collected a month after emergence traps were collected, indicating the importance of conducting these detection methods during the peak period for phylloxera abundance.

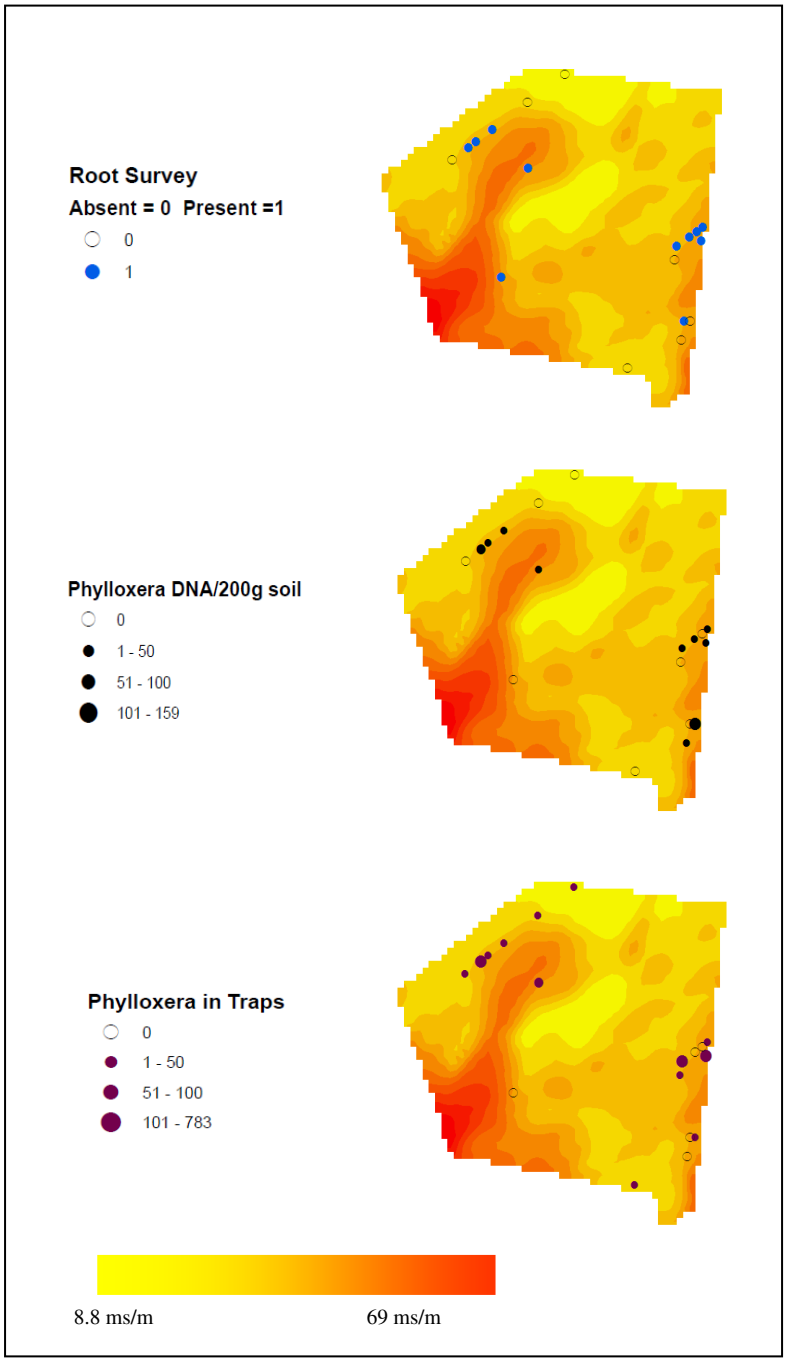


Figure 66 **An electromagnetic induction (EM38) survey of Block A (site 1) comparing the relative effectiveness of ground survey (March 2010), soil-based DNA testing (March 2010) and emergence trapping (January–February 2010).** *Open circles indicate no phylloxera was detected. Filled circles indicate phylloxera present. All phylloxera-positive samples were detected within the 14.2-28 mS/m range.*

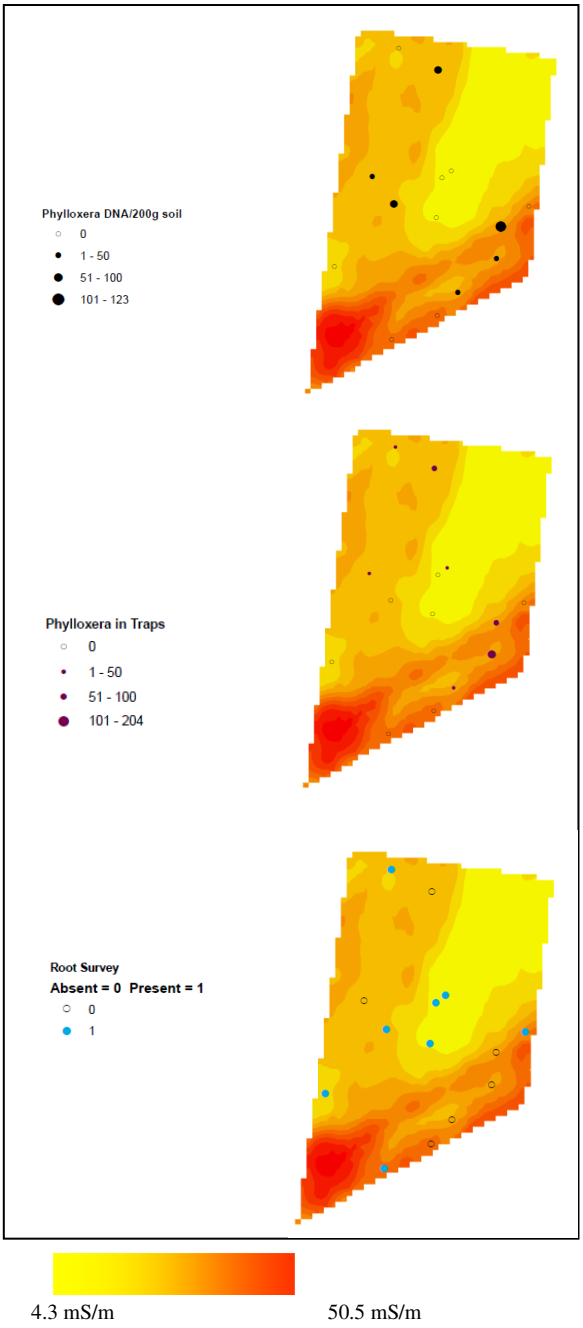


Figure 67 An electromagnetic induction (EM38) survey of Block D (site 1) comparing the relative effectiveness of ground survey (March 2010), soil-based DNA testing (March 2010) and emergence trapping (January –February 2010). *Open circles indicate no phylloxera was detected. Filled circles indicate phylloxera was detected. All phylloxera-positive samples were detected within the 17.5-19.5 mS/m range.*

Comparison DNA Probe - winter versus summer sampling - Season 2 (2009/2010)

To test the efficacy of using the DNA probe in the winter months a random selection of 18 core samples from infested vines and one from an uninfested vine (as assessed by DNA probe summer sampling in March 2010) were collected and screened in August 2010. Results indicated that overall the DNA probe is less effective when used in winter months compared to summer months (Fig. 68). In summer 2010, 18 positive samples were recorded whereas only 6 of the 18 vines were positive in the winter sample.

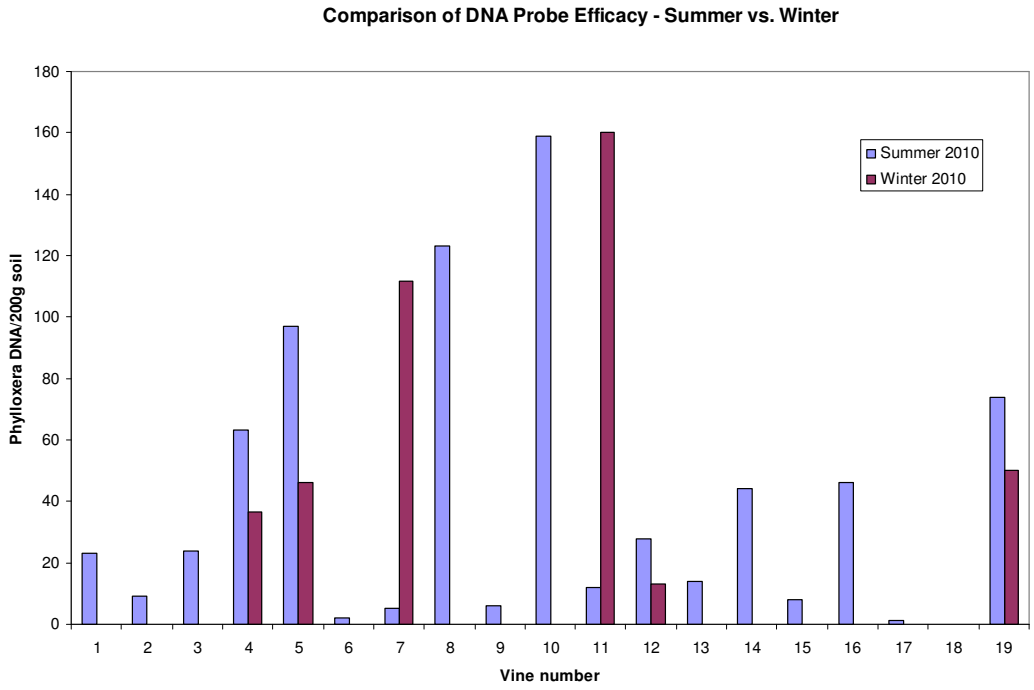


Figure 68 Comparison of soil phylloxera DNA probe efficacy from samples collected in summer (March 2010) and winter (August 2010) at site 1 in blocks A and D.

Comparative Detection Study - Season 3 (2010/2011) - Site 1

Figures 69-70 show maps of soil electrical conductivity derived from EM38 surveys of Blocks A and D, ground survey data, probe detected levels of phylloxera per 200g soil and in emergence traps. By season three phylloxera had clearly spread further than in the two previous 'early' detection seasons but in general remained within fairly narrow Eca ranges.

Detections by the survey team were similar to those detected by DNA testing, with both being higher than detections by the emergence trapping. Root surveying in season three was more effective than in the subsequent season which may be in part due to (i) increased spatial distribution and higher abundance of phylloxera compared with previous seasons and (ii) higher summer air temperatures reducing the emergence of phylloxera above-ground (and hence also reducing the sensitivity of emergence traps in this season) and therefore retaining higher populations on the root system. Emergence traps were marginally less sensitive in the third season which may be indicative of environmental factors affecting phylloxera emergence.

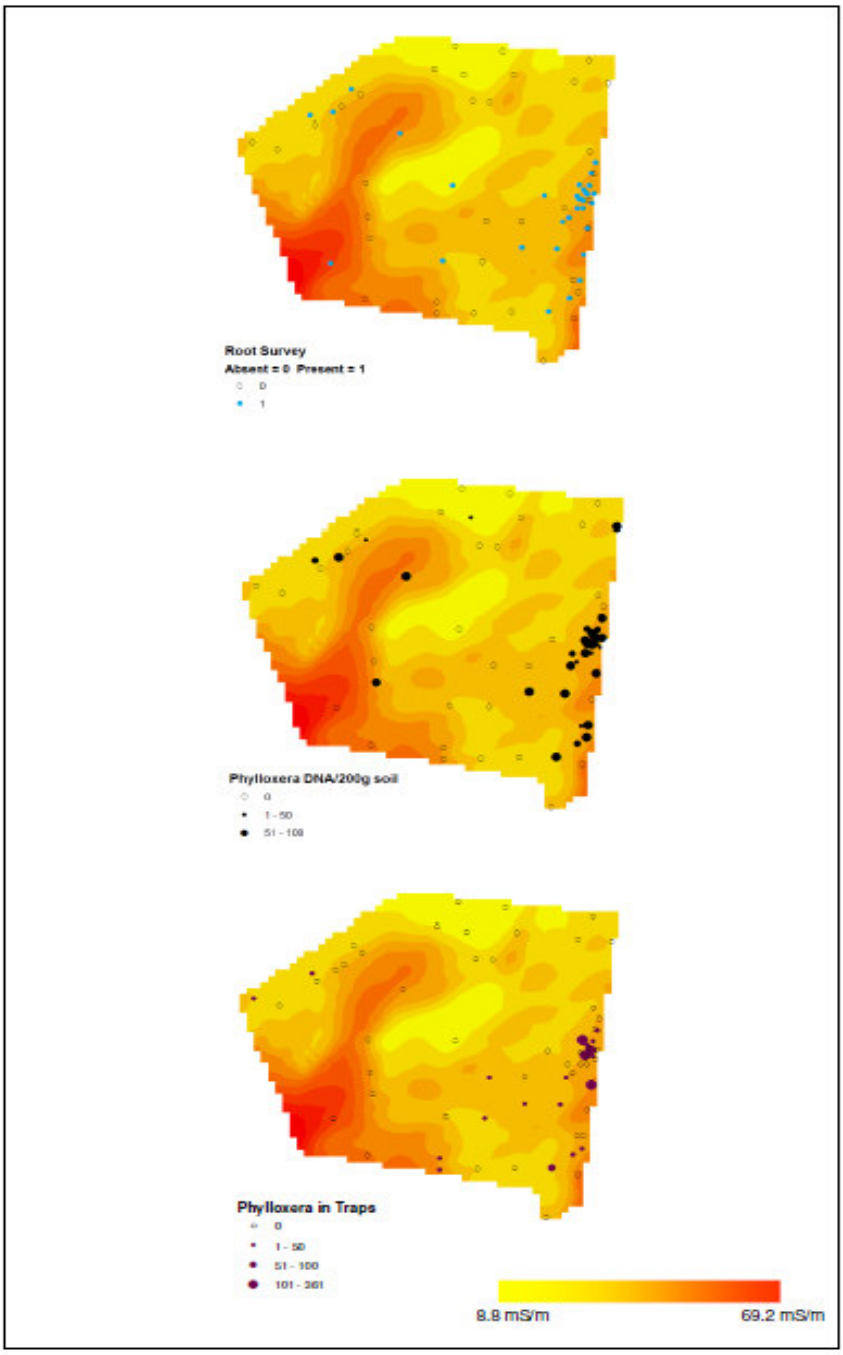


Figure 69 **An electromagnetic induction (EM38) survey of Block A comparing the relative effectiveness of ground survey (February 2011), soil-based DNA testing (March 2011) and emergence trapping (January–February 2011).** *Open circles indicate no phylloxera was detected. Filled circles indicate phylloxera was detected. Phylloxera-positive samples were predominantly detected within the 12.1-22.7 mS/m range.*

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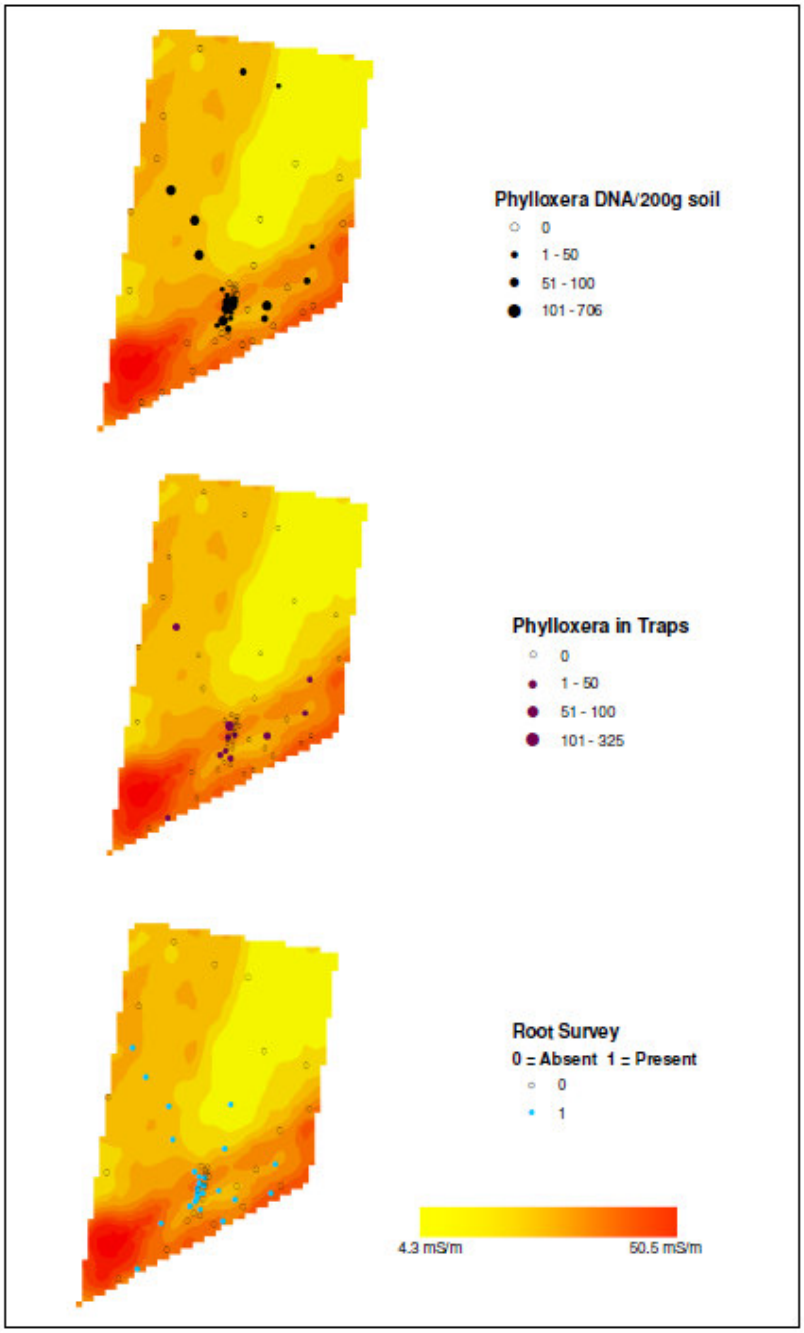


Figure 70 **An electromagnetic induction (EM38) survey of Block D comparing the relative effectiveness of ground survey (February 2011), soil-based DNA testing (March 2011) and emergence trapping (January–February 2011).** *Open circles indicate no phylloxera was detected. Filled circles indicate phylloxera was detected. All phylloxera-positive samples were detected within the 5.9-28.0 mS/m range.*

Targeted detection study – Phylloxera risk vineyard

An EM38 survey conducted at Site 2 in December 2011 showed EC values in the range of 21-88mS/m. An ECa range of 21-39mS/m, based on data selected from the *Comparative Detection Study* at site 1, was selected to conduct a pilot study of a targeted surveillance approach using only EM38 and emergence trapping. Traps were set in four blocks of ungrafted *V. vinifera* and sampled after 3 weeks. In one of the four blocks phylloxera was detected. Seven traps were set in Block Y within the lowest EC range 21-39mS/m in early December 2011. Three weeks post-placement the traps were sampled and phylloxera, if present, were counted. Phylloxera was detected in Block Y, a significant distance away from the original infested block (Fig. 71), in the risk vineyard site (Table 15) in 5 of the 7 traps placed within the block.

Table 15 Phylloxera detected in high risk vineyard as assessed using emergence traps in December 2011-January 2012.

Trap location (Block Y)	Total phylloxera
Row 4	5
Row 6 Vine 152	37
Row 6 Trap 2	38
Row 7	243
Row 8	1

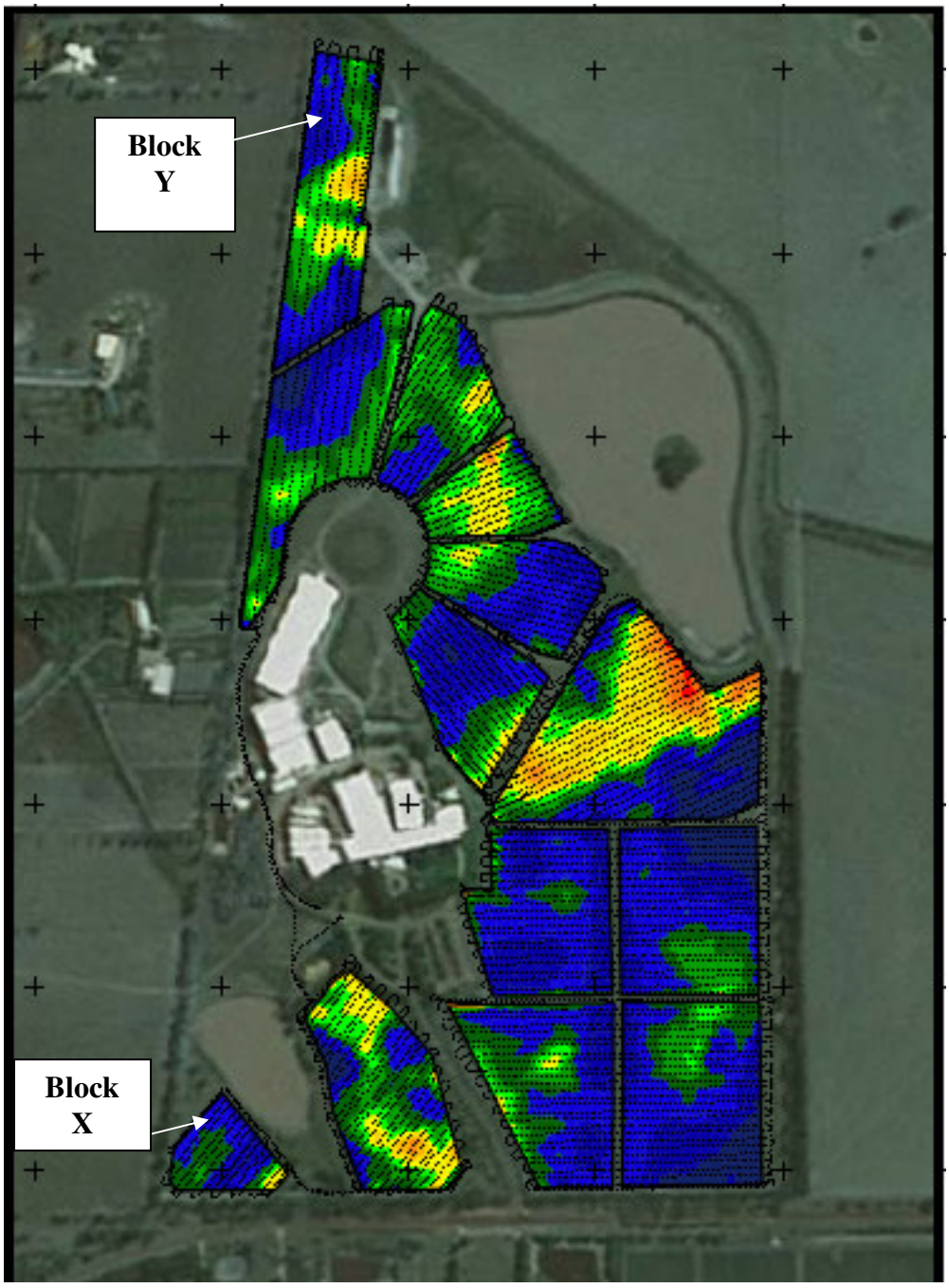


Figure 71 An electromagnetic induction (EM38) survey of whole vineyard (Site 2), in Yarra Valley region, conducted in December 2011.

Blue regions indicate medium range ECa range 21-39m/sm. Phylloxera was originally detected in block X (Bottom LHC) in December 2010, vines were removed and targeted trap emergence sampling conducted in December 2011-January 2012 in risk areas - detected phylloxera in Block Y (Top LHC).

Discussion

All methods of primary phylloxera detection used in this study, emergence traps, conventional surveys and DNA probe, were able to detect the insect particularly when used in summer months. This optimal time for sampling coincides with the highest level of population abundance (as assessed by seasonal emergence trapping). During the course of the project and at the request of local growers a simple protocol for phylloxera monitoring using emergence traps was developed (Appendix 2).

Delaying sampling by one month appeared to markedly reduce the effectiveness of the DNA probe. In winter even though the DNA probe could still detect phylloxera, winter sampling is not recommended, as it was considerably around 75% less effective compared to summer months. In the first two seasons of this study, at site 1, when the spatial distribution of phylloxera and population levels were relatively low both the DNA probe and the emergence traps were marginally more effective than the ground survey technique. Indicating they are likely to be more effective as 'early' detection tools where it may be difficult to observe weakened vines.

By the third season the root survey appeared as effective as the DNA probe and more effective than trapping. In the third season of the trial phylloxera-weakened vines were evident visually throughout both blocks which would make the survey teams location of phylloxera-infested vines far easier. However by this time the grower would need to be making decisions regarding replanting and detection at this point could not be regarded as ‘early’. The reasons for fewer positive trap samples are likely to be due to excessively damaged root systems and less abundant populations emerging from severely weakened vines.

The potential for further validation of targeted surveillance systems was highlighted when a pilot study conducted in a high risk vineyard (Site 2), using strategically placed emergence traps within EC ranges identified previously at Site 1. With minimal emergence

trap placement phylloxera was successfully detected in a previously unidentified infested block.

Recommendations

1. Due to financial constraints (i.e. each DNA sample costing $20-50 for laboratory processing) only a limited number of samples could be collected from the trial sites to allow detailed comparison of the soil DNA probe with conventional surveying and emergence trapping as detection tools. Even though results indicate that both emergence trapping and the soil DNA probe are relatively effective detection tools compared to conventional ground surveying, a more extensive study is required at multiple locations to effectively evaluate and develop an optimal integrated detection system. In particular further evaluation of the EM38 survey system as a secondary system, combined a primary detection system such as emergence trapping, is required as ultimately its use may allow for the development of a more targeted, less labour intensive and more economically viable detection and surveillance protocol.

2. The comparative phylloxera detection study has shown some extremely promising results, however the study was conducted on only two commercial vineyards in one grape-growing region and in the presence of a single highly virulent (and hence more readily detectable) phylloxera strain. It is strongly recommended that further studies should examine the efficacy of both the soil DNA probe and emergence traps, combined with EM38 surveys, at multiple time points within the season, under a range of soil and climatic conditions. These proposed studies should be conducted in both grafted- and ungrafted phylloxera-infested vineyards and preferably in vineyards which may also harbor low virulence, and hence potentially more difficult to detect, phylloxera genotypes.

3. Even though in this study the phylloxera DNA probe and emergence traps coupled with EM38 surveys appear to improve overall the sensitivity of phylloxera detection an economic analysis of the use of non-targeted and targeted surveillance techniques will be required, following multi-site comparison of detection systems, before national implementation of any new or revised phylloxera survey protocol can occur.

CHAPTER 7

PHYLLOXERA EDUCATION AND AWARENESS ACTIVITIES

***Output*:** *Knowledge transfer via National Phylloxera Workshops*

Summary

Nationally accredited Phylloxera Identification and Management Workshops have provided an essential service to the Australian viticulture industry since they were first instigated by DPI Victoria 16 years ago. Over 1500 participants have attended the courses to date (Fig. 72).

In this project, between January (2009) to December (2011), a total of 10 phylloxera workshops and ten half-day information sessions were delivered to over 600 participants from four states. There were three types of workshop formats depending on the target audience and location. Two-day workshops (organised in collaboration with The Phylloxera and Grape Industry Board of South Australia), one-day workshops (organised with either the Yarra Valley Grape Growers Association or Biosecurity Victoria) and half-day seminars held at DPI Victoria-Rutherglen Centre.

Workshop evaluation using KeePad™ technology an interactive, computer-based data collecting system was used at a range of workshops (Powell *et al*., 2009). In addition the project leader presented at six Phylloxera Biosecurity updates (200+ attendees) held in South Australia and Victoria (in association with Treasury Wine Estates). Both nationally (Victoria, South Australia, Queensland, ACT, New South Wales and the Northern Territory) and internationally (China, Austria and Armenia) the project leader was also invited to present at a number of conferences.

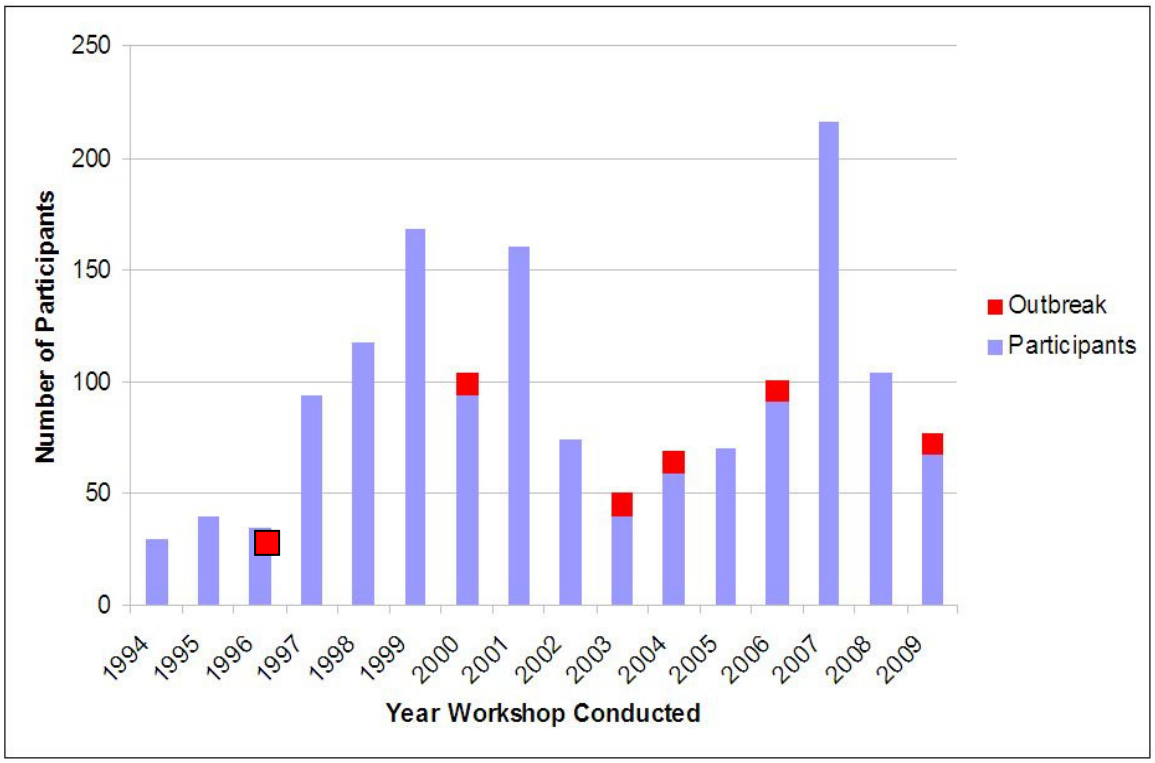


Figure 72 Phylloxera workshop attendance numbers since their commencement in 1994.

Red boxes in some years represent when phylloxera detections occurred, and highlight the corresponding changes to participant levels.

Introduction

Education and awareness form an integral part of any National Phylloxera Management Program. Phylloxera Identification and Management Workshops are designed to deliver information to the viticulture industry, and related personnel, about phylloxera management, including rootstock selection, disinfestation protocols, detection methods (Fig. 73) and quarantine to prevent the spread of phylloxera.

Although these workshops were primarily developed for and delivered to members of the viticulture and oenology industry, we felt the need to diversify and to reach a broader audience to enhance awareness of phylloxera risks. During the three-year period several one- and two-day workshops were organised along with half-day phylloxera awareness activities. These half-day sessions were mostly at the request of visitors to the DPI Victoria-Rutherglen Centre from different community organisations including:

- Alpine Valleys Leadership group (April 2011; 18 participants),
- Alpine Valley Agribusiness Leadership group (May 2011; 20 participants)
- Probus group (August 2010; 25 participants)
- Local high schools (July 2011; 45 students)
- Sydney University agriculture undergraduates (September 2010; 60 students)



Figure 73 Workshops attendees experiencing phylloxera detection in the field.

Method

The audience and level of background knowledge for the Phylloxera Management Workshops is so diverse that there was a need to deliver the message tailored to the audience using three different formats:

Comprehensive workshop format

In our well developed workshop program we have delivered high quality learning activities to a wide diversity of industry and educational personnel. The following ten topics were covered in the workshop and seminar programmes:

1. **Phylloxera biology, geography and history**
2. **Phylloxera genetics and interactions with rootstocks**
3. **Genotype population dynamics and distribution**
4. **Quarantine and risk management**
5. **Detection methods**
6. **Disinfestation facilities**
7. **Field survey and grower's talk**
8. **Phylloxera in the laboratory**
9. **Hypotheticals**
10. **Research update**

The one-day and two-day workshops were advertised nationally through viticulture industry journals (Australian and New Zealand Grapegrower and Winemaker, National Grapegrower and Australian Viticulture) and locally through regional grower associations. Participants were predominantly those personnel directly involved in the viticulture industry.

Vineyard survey workshop format

Participants for this workshop included contractors engaged to conduct phylloxera surveys as part of a PEZ rezoning program. This was a shortened version of the comprehensive workshop format, mostly focusing on the vineyard survey technique, quarantine protocols and highlighting the risks of phylloxera transfer. Every participant was trained to identify phylloxera symptoms and the insect in an infested vineyard.

Visiting group format

DPI-Rutherglen Centre attracts various visiting groups that are interested in state-of-the-art industry relevant science. To these groups a 30-60 minute overview was delivered followed

by a practical look-and-see session. The practical session included showing phylloxera under the microscope and a short update on phylloxera research activities and risk awareness.

These visiting groups included the following groups:

1. Alpine Valleys Community Leadership Group – a leadership program for community development.
2. Alpine Valley Business Group
3. Educational institutions – including high school students from Rutherglen High School and viticulture students from Charles Sturt University

Results

Over the three-year duration of the project 20 workshops were conducted and over 600 people were educated on phylloxera to different levels (Table 16). Workshop locations were chosen where there was either an infested vineyard so the participants gained hands on experience of phylloxera or at DPI Victoria-Rutherglen Centre where phylloxera populations are kept under controlled quarantine conditions.

Table 16 Participation levels and locations of Phylloxera Workshops in the three year project period (December 2008-December 2011).

	Dec 2008-Jan 2009	*2010*	*2011*	*TOTAL*
No. of workshops	5	5	10	14
Workshop locations	Yarra Valley	Milawa, Yarra Valley, Porepunkah, Rutherglen	Yarra Valley Rutherglen Heathcote Mildura South Australia	6
No of attendees	133	211	286	630

Evaluation process

Since late 2007 KeePad™ Technology, an interactive, computer based data-collecting system was introduced for phylloxera workshop evaluation. The level of effective learning at workshops has since been continuously monitored initially from using a paper based assessment (pre 2007) to a software-based assessment using an ARS (Audience Response System) from 2007-2010 (Fig. 74). The advent of KeePad™ and Turning Point™ technology provided an effective means to replace self-assessment. This technology also enabled mid-workshop adjustments to areas of the course that may be lacking a participant's comprehension. Using these advanced assessment methods also allows the employment of impact evaluation techniques. These ARS assessments have shown, on average, a 50% increase in obtained knowledge upon the completion of the workshop course (Fig. 75).



Figure 74 Keypads used to assess pre- and post-phylloxera knowledge at National Phylloxera Management and Identification Workshops.

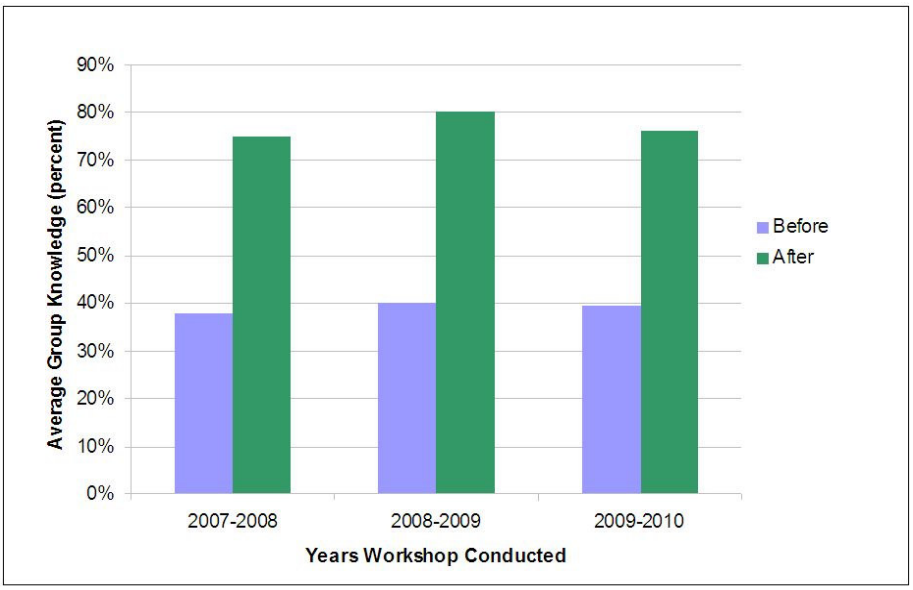


Figure 75 Measurement of phylloxera knowledge as assessed using an Audience Response System, both before and after the workshop, showing an average 50% increase in phylloxera knowledge of workshop participants.

National and International Presentations and Information Distribution.

In addition to workshops, industry articles, scientific papers and book chapters, conference presentations (both nationally and internationally (Fig. 76) have all ensured phylloxera awareness is maintained and the latest research outputs are provided to a range of stakeholders in the Australian viticulture industry (Appendix 1).

International Collaboration

In 2010 Dr Powell was invited by researchers at INRA Bordeaux and the University of Vienna to be the Australian representative on a proposed new international initiative to sequence the genome of the grape phylloxera (Delmotte *et al*., 2011). This could ultimately lead to major advancements in future management of grape phylloxera both nationally and internationally.



Figure 76 Shandong University Workshop attendees examining phylloxera and nematode galling on grapevine roots.

Discussion

Workshop evaluation using ARS systems have demonstrated that phylloxera workshops are an effective way of increasing phylloxera awareness. Phylloxera extension and awareness activities in an adaptable format are very important part of phylloxera management. During the project attendance levels were higher (600 + attendees) to those held in the preceding 3 years (400 attendees).

In 2009-10 phylloxera outbreaks within Victoria highlighted the need to educate more viticulture and oenology industry people and a wider public on phylloxera management and risks.

Recommendations

1. Phylloxera extension activities played a key role in both nationally and internationally and Australia is seen as a leader and innovator in this area. The increased number of participants to the phylloxera education and awareness activities shows that there is an ongoing need to continue with these activities. Reduction or indeed cessation of these activities may ultimately have a detrimental impact on the level of phylloxera awareness in the industry.
2. Recent phylloxera outbreaks in the Yarra Valley, and Mansfield highlighted the fact that more needs to be done in phylloxera awareness and rapid extension response. Post-phylloxera detection is also required as evidenced by the increasing number of phylloxera workshops conducted during the project period following detections in these regions.

APPENDIX 1: COMMUNICATIONS

Presentations were made at the following national and international industry and scientific conferences:

1. Australian Entomological Society 40th Annual General Meeting and Scientific Conference, Darwin, NT, 26-30 September 2009.
2. Global Biosecurity 2010: Safeguarding Agriculture and the Environment 28 February – 3 March 2010, Brisbane, Queensland.
3. Phylloxera: Trends, Practices, and Improvements in Armenia. 2-3 June 2010, Armenia.
4. The 14th Australian Wine Industry Technical Conference, 3-8th July-August 2010, Adelaide, South Australia
5. The 5th International Phylloxera Symposium, Vienna, Austria, 9-23 September 2010 (See Powell *et al*., 2010).
6. Australian Entomological Society 41st Annual General Meeting and Scientific Conference, Perth, WA, 26-30 September 2010.
7. ASVO Conference on Below Ground Management for Quality and Productivity Seminar. 28-29th July, Mildura, 2011.
8. Phylloxera Workshop, Shandong University, China May 2011.
9. Multiple presentations to Treasury Wine Estates, South Australia and Victoria, October 2011

Involvement with Working groups/Reference groups:

1. Technical Adviser to National Phylloxera Technical Reference group.
2. Entomology Adviser to Plant Health Australia on Industry Biosecurity Group reviewing and revising the National Viticulture Industry Biosecurity Plan.

3. Australian representative on International Phylloxera Genomics Initiative committee.
4. Invited Scientific Advisor for 5th International Phylloxera Symposium, Vienna, 2010.

Awards and recognition:

The following awards where presented to the Phylloxera research team during the course of the project:

2008 – Winner of DPI Aristotle Award for Best PhD Student. Dr Kim Kingston.

Papers were published in peer-reviewed scientific journals, conference proceedings, book chapters and industry journals as follows:

<u>Referred publications 2009-2012</u>

Andrews KB., Cooper PD., Kemper D., Powell KS. 2012. Spatial trade-offs in the digestive and reproductive systems of grape phylloxera. *Australian Journal of Zoology* **59,** 392-399.

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Industry Articles:

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Powell, KS., Korosi, GA, Carmody, B, Bruce, R. 2012. Impacts of soil electrical conductivity and compost mulches on phylloxera establishment and abundance. *Proceedings of ASVO Conference on Below Ground Management for Quality and Productivity Seminar* 28-29th July, Mildura.

APPENDIX 2: TRAP PROTOCOL

Emergence traps for monitoring soil-borne insects





K.S. Powell – DPI, Biosciences Research – Bioprotection, RMB 1145, Chiltern Valley Rd, Rutherglen, VIC 3685

Some soil pests, including root galling phylloxera, emerge from the soil in the spring and summer months. By inverting a plastic container (emergence trap) on the soil surface some of these pests can be trapped in the condensation as they crawl up the sides of the trap. Once trapped insects can be collected for identification.

Equipment required:

- Translucent round plastic container (4.0 to 5.1 litre capacity).
- Claw hammer
- 3 tent pegs (per trap)
- Water
- Methanol or 70% ethanol
- Squirt bottle

Trap placement (see Figures 1-6):

Traps are best placed in January or February to optimise the number of insects collected. However they can also be used at anytime between December to March.

1. If a weak patch of vines is evident, place traps next to a healthy vine at the edge of the weak spot. Prior to trap placement clear mulch or plant material and level the soil surface, with claw hammer, adjacent to grapevine trunk.

2. Rinse the plastic container (ie. emergence trap) with a small volume of water (about 200ml) and discard excess leaving a thin film of moisture.

3. Secure the inverted container on the soil surface using tent pegs and hammer. Ensure that the container edges are well sealed so that condensation forms in the trap over a 1-2 hour period.

4. After 2-4 weeks, rinse the contents of the container using a squirt bottle containing methanol or 70% ethanol, ensuring collection of moisture from all sides, into a screw cap plastic container.

5. In a secure area, label each sample jar clearly both inside and outside the container with a marker which doesn't wash off in ethanol. The label should show collection date, vineyard name, row number and vine number.

Sample identification:

Emergence traps do collect a diverse range of insect types which to the untrained eye may look similar to phylloxera. It is therefore essential that they are examined by a qualified entomologist.

1. To ensure accurate diagnosis transport the sample securely to an accredited laboratory (DPI-Rutherglen or DPI Knoxfield) **with appropriate permit** (obtained from nearest Plant Standards branch) for identification or alternatively contact DPI Rutherglen for collection. There is a charge for sample identification.

2. Samples can be transported by courier or express mail. However, please note Australia Post and courier regulations for transport requirements of flammable liquids.



(1) Emergence trap adjacent to grapevine trunk.



(2) Trap rinsed with water.



(3) Trap, secured with tent pegs, showing condensation.



(4) After 2-4 weeks remove trap and rinse with 70% ethanol or methanol.



(5) Collect sample in screw top plastic vial.



(6) Trap is rinsed in water and repositioned if further samples required.

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APPENDIX 3: INTELLECTUAL PROPERTY

APPENDIX 4: BIBLIOGRAPHY

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APPENDIX 5: STAFF

Project Activities

The projects core activities were carried out by DPI-Rutherglen staff. Phylloxera and *Vitis* genotyping was conducted by staff at CESAR, University of Melbourne. The project activities were carried out in close collaboration with local growers in North East Victoria, the Phylloxera and Grape Industry Board of South Australia, the National Phylloxera Technical Reference Group and CSIRO Merbein.

Project Staff

DPI-Rutherglen staff involved in the project activities included Dr Kevin Powell (Project Leader), Ginger Korosi, Rebecca Bruce, Peter Mee (Graduate Program) and Bernadette Carmody.

Adherence to Quarantine Protocols

All fieldwork was carried out under permits that specified procedures and disinfestation protocols designed to prevent the spread of phylloxera by project staff. Field visits were arranged in consultation with vineyard managers. All field equipment, clothing and footwear was cleaned and disinfested after visiting infested vineyards.

Field collected samples were stored and transported in sealed containers under permit from the vineyard to laboratory facilities at DPI Rutherglen Centre. All field collected samples were processed in laboratory facilities at DPI Rutherglen Centre which is located within the North-East Victoria Phylloxera Infested Zone (PIZ). Insect and plant materials which required genetic characterisation were transferred under quarantine regulations under permit issued by Plant Standards-Victoria.

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APPENDIX 6: BUDGET RECONCILIATION